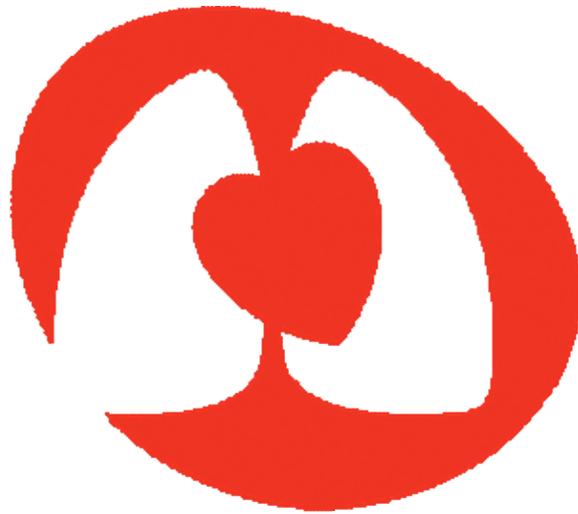


National Heart, Lung, and Blood Institute
Division of Intramural Research

***Eighth Annual NHLBI DIR
Scientific Retreat***



April 14-16, 2010
Tremont Suite Hotel
Baltimore, Maryland

2009-2010 Fellows Advisory Committee

Joshua Anzinger Translational Medicine Branch	Cory Lago Translational Medicine Branch	Aibin Wang Laboratory of Molecular Cardiology
Kjetil Ask Translational Medicine Branch	Jonathan Lam Translational Medicine Branch	Lu Wang Laboratory of Molecular Immunology
Arianna Biesso Laboratory of Cell Biology	Yuan Le Laboratory of Cardiac Energetics	Thomas Winkler Hematology Branch
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Kang Chen Laboratory of Biophysical Chemistry	Attila Nagy Laboratory of Molecular Physiology	Zheng You Laboratory of Biochemistry
Ashvin George Laboratory of Biochemistry	Yrina Rochman Laboratory of Molecular Immunology	Yingfan Zhang Laboratory of Molecular Cardiology
Mohit Kashyap Laboratory of Molecular Immunology	Mark Stevens Translational Medicine Branch	Hang Zhao Laboratory of Biochemistry

2009-2010 Staff Scientist Committee

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Jason Hoffert Laboratory of Kidney and Electrolyte Metabolism		Ashok Srinivasan Genetics and Developmental Biology Center

With Special Help From
The DIR Office of Education
Office of the Scientific Director
Food and Drug Administration
NHLBI Cores and Offices

**Eighth Annual NHLBI DIR Fellows Retreat
Baltimore, Maryland
April 14 - 16, 2010**

Wednesday, April 14th, 2010

- 4:00 **Arrival, Registration, Check-In – *Edinburgh Hall***
- 4:30 – 7:00 **Poster Session I – *Edinburgh Hall***
- 7:00 – 8:00 **Dinner – *Marble Room***
- 8:00 – 8:15 **Introduction & Welcome – *Mirror Room***
Chair: Yrina Rochman, Ph.D.
Fellows Advisory Committee Members
Staff Scientist Committee Members
Herbert M. Geller, Ph.D., Director, Office of Education
Robert S. Balaban, Ph.D., Scientific Director, NHLBI
- 8:15 – 9:30 **Postdoctoral Career Presentations – *Mirror Room***
Robert A. Burton, Ph.D., Principal Invest., BAE Systems, Technology Solutions
Jennifer A. Hobin, Ph.D., Assoc. Dir for Scientific Affairs, Office of Public Affairs,
Federation of American Societies for Experimental Biology (FASEB)
Fraser D. Brown, Ph.D., Attorney, J.D., Banner and Witcoff, Ltd.
- 8:15 – 10:00 **PI/Staff Scientist Social – *Composite Room***

Thursday, April 15th, 2010

- 8:00 – 9:00 **Breakfast and Registration – *Marble Room***
- 9:00 – 10:15 **Scientific Speaker – *Mirror Room***
Chair: Kjetil Ask, Ph.D.

Peter Walter, Ph.D., Professor, UCSF
Investigator, Howard Hughes Medical Institute
- 10:15 – 10:30 **Group Picture**
- 10:30 – 12:00 **Research Highlights, Session I – *Mirror Room***
Chair: Cory Lago, Ph.D.

Wenling Li, Ph.D., Visiting Fellow, Lab of Developmental Biology,
CXCL12-CXCR4 Signal Regulates Nerve-Mediated Arterial Branching in the
Developing Limb Skin

Jamie Schroeder, Ph.D., Predoc Fellow, Translational Medicine Branch
In vivo Multiphoton Imaging Using a Motion Compensating Microscope:
System Design and Novel Observations

Allen Chang, Ph.D., Research Fellow, Laboratory of Biochemistry,
A Non-Proteasomal Catabolic Pathway for Oxidized Protein-IRP2 as a
Model System

Heather Lucas, Ph.D., IRTA Fellow, Laboratory of Molecular Biophysics
Copper(I) and Copper(II) Binding to ∞ -Synuclein

12:00 – 1:30

Lunch

Practice your “Elevator Talk” (Refer to page 7)

Poster Session II Mounted

1:30 – 2:30

Free Time

2:30 – 4:00

Research Highlights, Session II – *Mirror Room*

Chair: Ashvin George, Ph.D.

Andrew Johnson, Ph.D., Research Fellow, Center for Population Studies
Multiple Platelet Aggregation Genes are Identified by Genome-wide Association
Meta-analyses

Zhibin Wang, Ph.D., Visiting Fellow, Immunology Center
How Do Corepressor HDACs Act in the Human Genome

Jean-Cheng Kuo, Ph.D., Visiting Fellow, Laboratory of Cell and Tissue
Morphodynamics
Proteomic Analysis of Myosin II-mediated Focal Adhesion Maturation

Wolfgang Wagner, Ph.D., Visiting Fellow, Laboratory of Cell Biology
The Class V Myosin MYO5A Transports the Endoplasmic Reticulum into
the Dendritic Spines of Purkinje Neurons

4:00 – 6:30

Poster Session II - *Edinburgh Hall*

Representatives from FDA

Betty Clark, Recruitment Specialist, Center for Drug
Evaluation and Research

B. Mary Heckler, Recruitment Specialist, Center for Drug
Evaluation and Research

Owen McMasters, Ph.D., Pharmacologist, Office of Antimicrobial Products

6:30 – 7:30

Dinner – *Marble Room*

8:00 – 9:30

Featured Speaker – *Mirror Room*

Chair: Mark Stevens, Ph.D.

Robert P. Kocher, M.D., Special Assistant to the President,
National Economic Council

Friday, April 16th , 2010

7:30 – 8:30 **Breakfast – *Marble Room***

8:30 – 10:00 **Scientific Speaker – *Mirror Room***
Chair: Zheng You, Ph.D.

Gerald I. Shulman, M.D., Ph.D., Professor, Yale University
Investigator, Howard Hughes Medical Institute

10:00 – 11:30 **Research Highlights, Session III – *Mirror Room***
Chair: Yuan Le, Ph.D.

Rodrigo Calado, M.D, Ph.D., Research Fellow, Hematology Branch
Telomere Dysfunction in Human Disease

In Hye Lee, Ph.D., Visiting Fellow, Translational Medicine Branch
The Essential Autophagy Gene Atg7 Coordinates Cell Cycle Withdrawal and
Survival with Nutrient Status

Xianglan Yao, Ph.D., Staff Scientist, Pulmonary and Vascular Medicine Branch
An Apolipoprotein E - LDL Receptor Pathway Regulates the Pathogenesis of
House Dust Mite Induced Asthma

Ho Joong Sung, Ph.D., Visiting Fellow, Translational Medicine Branch
Mitochondrial respiration Protects Against Oxygen-associated DNA Damage

11:30 – 12:00 **Awards Ceremony – *Mirror Room***
Chair: Yingfan Zhang, Ph.D.

Fellows Poster Awards
Fellows Award for Research Mentoring

12:00 – 1:00 **Closing Banquet – *Marble Room***

2:00 **Departure**
Buses meet at main entrance at 2:00 p.m.

Practice your “Elevator Talk” for Postdoctoral Fellows

Imagine that you are at a scientific meeting waiting for an elevator at one of the big convention hotels. And the doors open and you find yourself face-to-face with the major figure in your field that you’ve been wanting to meet. And your poster presentation is the next day? What do you do next?

What you do is give your elevator talk - a carefully thought-out summary of your scientific interests and achievements, something that you can deliver to a complete stranger in 30-seconds or less (the time it takes to share small talk with someone in an elevator who says, “So tell me, what is it you do?”), presented in a way that will entice Dr. Bigshot to come to your poster. And you have just the duration of an elevator ride to give it!

This is the object of our lunch on Wednesday, April 15th. You will be assigned to a table for lunch, along with other fellows from other labs, along with 1 or 2 NHLBI PIs. Your objective is to present yourself, including your background, significance, and future goals, in a 3 minute talk to all at your table. There will be enough time for you to receive feedback from the group

Poster Awards for All

Awards will be given for the best poster presentations. There are separate categories for post-docs, staff scientists and post-bacs and graduate students. Posters will be judged by members of the Fellows Advisory Committee and NHLBI Tenure-Track Investigators. The criteria include the scientific content of the poster, the organization of the poster, as well as the presentation skills of the poster presenter. If you wish to be judged, please be with your poster at the time assigned on the program.

Scavenger Hunt for Post-baccalaureates

This scavenger hunt is designed to be a **fun** and **educational** activity for post-baccalaureate fellows. This exercise is designed to help answer the most frequently asked questions indicated on the post-baccalaureate survey that was administered last month. Please take the time to do the hunt because it will not only provide you with answers to the questions that you have, but it will increase your interaction with others within the NHLBI...besides, the **first 10 people** to pass in their completed form to **Angela or Aurora** will win a **great prize** and be presented with it at the Awards Ceremony on Friday. Hint: most of these items can be completed during the poster presentations.

1. Visit **7 of the 14** NHLBI Core Facility Poster Presentations.
2. Speak with **5 post-doctoral fellows** (who are not in your lab)
3. Speak with 2 principal investigators (outside of your center or branch)
4. Find **one** person who uses the **NHLBI Take 10 Room**
5. Introduce yourself to another post-baccalaureate fellow that you do not know.
6. At the poster session, find somebody who has presented a technique that you are not familiar with or would like to learn how to do.

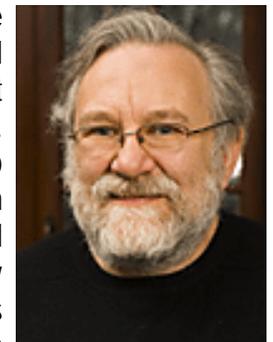
Speaker Biographies

Gerald I. Shulman, M.D., Ph.D., is Professor of Internal Medicine & Cellular and Molecular Physiology at Yale University, where he serves as Associate Director of the Yale Diabetes Endocrine Research Center and Associate Director of the Yale Medical Scientist (M.D., Ph.D.) Program. He is also an Investigator of the Howard Hughes Medical Institute. Dr. Shulman is an internationally recognized diabetes researcher, and the recipient of numerous awards, including the Outstanding Scientific Achievement Award and the Distinguished Clinical Scientist Award from the American Diabetes Association, the Diabetes Care Research Award from Boehringer-Mannheim/Juvenile Diabetes Foundation, and the Stanley Korsmeyer Award from the American Society for Clinical Investigation (ASCI). In addition to the ASCI Dr. Shulman has been elected to the Association of American Physicians, the Institute of Medicine and the National Academy of Sciences. He is a leading authority on the cellular mechanisms of insulin resistance, the role of the liver and muscle in the pathogenesis of type 2 diabetes, and the benefits of exercise in the management of diabetes. Dr. Shulman's practice is limited to the treatment of diabetes, and other disorders of glucose metabolism, with specific interests in the management of newly diagnosed type 2 diabetes mellitus.



Robert P. Kocher, M.D., is Special Assistant to the President for Health Care. An expert on international health policy and the economics of U.S. health care, he joined the Obama administration as a member of the National Economic Council. As a member of the president's health-care economics and policy brain trust, he is on the front lines of the administration's efforts to shape and enact health care reform. He is a former partner at the Washington-based international consulting firm McKinsey & Co., where he led research efforts to determine causes of high U.S. health-care costs for the McKinsey Global Institute, the firm's economic research department. He is an active writer and public speaker on a range of healthcare topics including healthcare reform, healthcare economics, improving clinical outcomes, and international healthcare strategies for US hospitals. He received his undergraduate degree from the University of Washington and medical degree from George Washington University. He completed a research fellowship with the Howard Hughes Medical Institute and the National Institutes of Health. He went on to complete his internal residency training at the Beth Israel Deaconess Medical Center and the Harvard Medical School. In his current role, he is charged with finding ways to reform the U.S. health system by slowing cost increases and improving clinical outcomes. He also advises the president on rural economic policy, food safety and obesity.

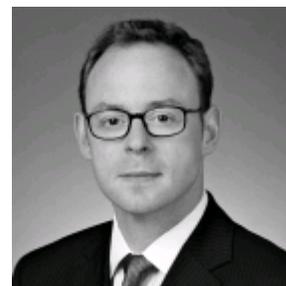
Peter Walter, Ph.D., is a Professor in the Department of Biochemistry & Biophysics at the University of California, San Francisco, and also an Investigator of the Howard Hughes Medical Institute. After earning a Vordiplom at Freie University, Berlin, Dr. Walter attended Vanderbilt University as an Exchange Fellow and was awarded a M.Sc. degree in organic chemistry. In 1982 Dr. Walter joined the faculty of the Department of Biochemistry & Biophysics at UCSF, where he has also served as Director of the Cell Biology Program and Co-Director of the Science & Health Education Partnership. Research in Dr. Walter's lab concerns organelle biogenesis, membrane dynamics, and protein sorting and focuses on such basic questions in cell biology as how proteins become properly localized within a cell, how cell membranes fuse, and how cells maintain the abundance of organelles in proper balance. It is this last question that led to the characterization of the unfolded protein response (UPR) pathway, an intracellular signaling pathway that allows the endoplasmic reticulum to communicate with the nucleus to regulate gene expression. Recent evidence suggests that the UPR is dysregulated in a number of disease processes and may therefore represent an important target for potentially useful therapeutic intervention for cancer and other diseases. Professor Walter is a co-author of *Molecular Biology of the Cell*, now in its fifth edition. He also is a recipient of the Gairdner Award, the Canadian equivalent of the Nobel prize.





Jennifer A. Hobin, Ph.D. is the Associate Director for Scientific Affairs at the Federation of American Societies for Experimental Biology (FASEB). At FASEB, Dr. Hobin works on issues related to scientific training and career development, clinical research, and peer review. Her duties include tracking and analyzing policy changes, developing policy recommendations, and producing communications materials and outreach tools. Prior to joining FASEB, Dr. Hobin was a Christine Mirzayan Science and Technology Policy Graduate Fellow at the National Academies' Committee on Science, Engineering, and Public Policy, where she contributed to a report on maximizing the potential of women in academic science and engineering. She earned her PhD in biopsychology from the University of Michigan by describing the neural circuits mediating the context-specific expression of Pavlovian fear memory. She has a BA in psychology, summa cum laude, from Stony Brook University. Dr. Hobin serves on the National Postdoctoral Association's Advocacy Committee and is

Fraser D. Brown, Ph.D., J.D., is an Attorney in the Washington, DC office of Banner & Witcoff, Ltd. Dr. Brown earned his Bachelor of Science degree in Biochemistry from the University of Glasgow, Scotland, and his Ph.D. in Biology (Cell Signaling) from the University of Birmingham, England. He received his J.D. from Georgetown University, attending law school at night while apprenticing during the day. Dr. Brown spent seven years at the National Institutes of Health in Bethesda, MD performing post-doctoral research in molecular biology and imaging as applied to membrane trafficking and cell signaling pathways. He attended law school at night and apprenticed for the firm during the day. Dr. Brown focuses his practice in the field of biotechnology, diagnostic and therapeutic methods, and therapeutic biologics. He conducts U.S. and foreign patent prosecution and renders patentability, non-infringement, invalidity, and freedom to operate opinions. Dr. Brown recently co-authored a guest column titled "Oral Argument Sheds Light in *Bilski v. Kappos*," published in the December 2009 issue of Genetic Engineering & Biotechnology News.



Robert A. Burton, Ph.D. is a Principal Investigator at BAE Systems, Technology Solutions in the Advanced Materials Group. His work focuses on detection and decontamination of chemical and biological threat agents and combat casualty care. He oversees several projects and works at the interface between government funding agencies, customer requirements, and product research, development and manufacturing. While a L'Enfant Biomedical Fellow at the NHLBI, NIH, he determined the structure and dynamics of the fibrinogen alpha-C domain and explored new approaches to utilizing chemical shift in protein structure determination. He received his Ph.D. in Structural Biology and Biophysics from Purdue University elucidating the structure/function relationships and dynamics of protein tyrosine kinases in key cell signaling pathways. He has a MS in Organic Chemistry and B.S. in Chemistry from Brigham Young University.

Animal MRI Core Facility

Stasia A. Anderson, Ph.D., Director

Building 10, Room 2N240, E-mail: andersos1@nhlbi.nih.gov

Phone: (301) 402-0908; Web: <http://dir-intranet.nhlbi.nih.gov/amri>

The Animal MRI/Imaging Core is a resource for biomedical imaging of small animal models in the NHLBI. The MRI Core develops and optimizes MRI methods for cardiovascular imaging of mice and rats. We provide imaging expertise, data interpretation and experimental design for investigators interested in incorporating imaging studies in NHLBI research. We are a teaching resource, and investigators and fellows can learn to perform MRI studies. Examples of imaging studies in the Core are:

- Cine cardiac imaging for ejection fraction, ventricle size and wall thickness
- High resolution imaging of myocardium for identification of infarct
- Imaging aorta and vessels in live mice and rats
- Imaging atherosclerotic plaque
- Perfusion of skeletal muscle
- Cellular imaging: magnetic labeling and tracking cell transplants
- Targeted MRI contrast agent research
- High resolution microimaging of embryos and fixed tissue

We work with investigators on the best approaches for the research model and goals. We can incorporate additional imaging modalities such as computed tomography, ultrasound and bioluminescence. Core imaging studies are performed in the NIH Mouse Imaging Facility.

The Biophysics Facility

Grzegorz (Greg) Piszczek, Ph.D. Facility Director

Building 50, Room 2341; E-mail: piszczek@nih.gov

Phone: (301) 435 8082; Web: <https://dirweb.nhlbi.nih.gov/Cores/BF/Pages/default.aspx>

The mission of the Biophysics Facility is to provide state of the art equipment and training to assist investigators within the NHLBI in studies of macromolecular interactions, dynamics and stability. The Biophysics Facility currently has resources to study oligomeric state of biomolecular assemblies, perform measurements of affinity, stoichiometry, kinetics and thermodynamics of interactions between proteins, DNA and their cognate ligands. Biophysical characterization capabilities include measurements of molecular weight, shape, and conformation of biological macromolecules.

Oligomeric state of macromolecular assemblies, including their size and shape, can be studied using both analytical ultracentrifugation (Beckman XLI and XLA) and light scattering techniques (Multi Angle Static and Dynamic Light Scattering – MALS, DLS). Most physical or chemical processes have an associated heat effect that can be used as the basis for a number of analytical techniques. Microcalorimetry is now the biophysical method of choice for non-invasive, label-free analysis of biomolecular interactions and stability. The Biophysics Facility has several Isothermal Titration and Differential Scanning Calorimeters (ITC and DSC) that can be used in those studies. Surface Plasmon Resonance (SPR) is a complementary method for studying macromolecular interactions that can also provide information on binding kinetics and Facility is equipped with the Biacore 3000 and 1000 instruments. Additionally, Facility users can take advantage of several optical spectroscopy methods, including steady-state and time resolved fluorescence, fluorescence anisotropy, circular dichroism and stopped-flow.

Electron Microscopy Core Facility

Mathew P. Daniels, Ph.D., Director

Building 14E, Room 111B, E-mail: danielsm2@mail.nih.gov

Phone: (301) 496-2898, Fax: (301) 480-6560

The NHLBI Electron Microscopy Core Facility offers consultation, technical services and training in electron microscopy to all investigators in the NHLBI/DIR. We have collaborated with many NHLBI investigators since the Core was formed in 2006 and in 2008 we worked with the research groups of 16 principle investigators. Our technical capabilities include: 1) Transmission electron microscopy and scanning electron microscopy of tissues, cells and other biological or material samples. 2) Immunocytochemistry at the electron microscopic level including immuno-gold labeling. 3) Negative staining and rotary shadowing of macromolecular preparations (nucleic acids or proteins) as well as other small structures such as viruses and liposomes. We offer training in the use of both the transmission electron microscope and the scanning electron microscope as well as in the interpretation of ultrastructure, and encourage postdoctoral fellows and students to perform image acquisition on their own samples.

Flow Cytometry Core Facility

J. Philip McCoy, Jr., Ph.D., Director

Building 10, Rooms 8C104, E-mail: mccoyjp@mail.nih.gov

Phone: (301) 594-6950, Fax: (301) 480-4774

The mission of the NHLBI Flow Cytometry Core Facility is to provide investigators at the NHLBI access to state-of-the-art flow cytometry. This is done by having cytometers and software available in the core facility and by providing consultation to investigators who have cytometers available in their own laboratories or branches. Investigators are responsible for specimen preparation and staining. The staff of the flow cytometry laboratory will gladly assist you in designing your experiments and in developing optimal preparation and staining procedures. For analytical experiments, data will be provided as either hard copies or on appropriate media as listmode files. FCSExpress software (DeNovo Software) will be available for "offline" analysis of these files. For sorting experiments, each investigator is responsible for bringing appropriate media and test tubes. In addition to cell sorting and analytical cytometry, the core facility also provides multiplex bead array expertise for analysis of extracellular cytokines, and imaging flow cytometry where the staining patterns of fluorochromes can be visualized.

Genomics Core Facility

Nalini Raghavachari, Ph.D.

Building 10, 8C103 B, 8C215; E-mail: nraghavachari@cc.nih.gov

Phone: (301) 435-2304

The purpose of the genomics core facility is to provide NHLBI investigators high quality, state-of-the-art gene expression profiling using Expression, Exon and miRNA arrays & genotyping services in a timely fashion using the Affymetrix platform. The core provides this high quality service by implementing rigorous standardization of protocols and multiple quality control checks at various points during sample processing and gene chip hybridization. We also provide integrated service to investigators in a collaborative manner for the design of microarray experiments, target preparation, streamlined data analysis applying complex statistical tools and validation of data by Taqman analysis on ABI 7900. Assistance will also be provided in the amplification of small amounts of RNA by applying our established amplification protocols.

Imaging Probe Development Center

Gary L. Griffiths, Ph.D., Director

9800 Medical Center Drive, Building 2B, Rockville MD

Phone: (301) 217 5770; E-mail: griffithsgl@mail.nih.gov

The Imaging Probe Development Center (IPDC) was founded as part of the 2003 trans-NIH Roadmap for Medical Research initiative with the aim of providing essential synthetic chemistry support needed to advance molecular imaging technologies in interdisciplinary research at basic through translational to clinical levels. The IPDC laboratories are located in Rockville, MD with state-of-the-art equipment and scientists drawn from diverse backgrounds with expertise in synthetic, inorganic, radiochemical, and conjugation chemistries. The IPDC has a rolling solicitation system and NIH scientists are welcome at any time to enquire about obtaining a probe in which they are interested. The IPDC is currently working with principal investigators from more than a dozen different NIH Institutes, including several from NHLBI. We can supply requested imaging probes that are already known but otherwise unavailable, or are completely novel, and probes can be intended for all types of imaging modalities, including MRI, optical fluorescence and PET/SPECT. Examples of molecular imaging probes we have made include various complexes for MRI studies, fluorogenic enzyme substrates, fluorescent dyes and analogs, caged derivatives which become fluorescent upon uncaging, radio- and fluorescent-labeled antibodies and other proteins, radiolabeled low molecular weight compounds, liposomes, dendrimers and nanoparticles. For more information, or to make inquiries about probe availability you may visit our website: <http://www.ipdc.nih.gov>, or please feel free to speak with an IPDC staff member about agents of interest to you.

Laboratory of Animal Medicine and Surgery (LAMS)

Robert F. Hoyt, Jr., DVM, MS, DACLAM, Chief

Bldg 14E, Rm 105A, E-mail: hoytr@nhlbi.nih.gov

Phone: (301) 496-9673, Fax: (301) 496-9673

Randall R. Clevenger, B.S., LATG, 14E Surgical Facility Manager,

Bldg 14E, Rm 106B, E-mail: rc85n@nih.gov

Phone: (301) 496-0405, Fax: (301) 402-0170

The Laboratory of Animal Medicine and Surgery (LAMS) Core provides veterinary medical care and technical services for NHLBI research animals used in both basic and preclinical research. These support services include: veterinary medical care, surgery, surgical support, investigator training in surgical and microsurgical techniques, post-operative care, purchasing (large animals), and health monitoring. In addition, LAMS provides technical services such as blood & tissue collection, tail vein injections, and has a clinical laboratory for determining hematology, urinalysis, and blood chemistries in research animals. The LAMS staff provides 24-hour emergency veterinary care.

The LAMS staff also provides NHLBI investigators with collaborative research support services such as developing animal models and new surgical procedures, assistance with research design, as well as animal protocol development and execution. Our surgical support equipment includes anesthesia machines with mechanical ventilation for species ranging from rodents to nonhuman primates, radiography, digital fluoroscopy, Faxitron, ultrasound, laser Doppler, and operating microscopes. Our full-service clinical laboratory is available for supporting research requirements.

LAMS supports a wide range of animal models for DIR investigators. Some of the animal models we provide surgical support for include xenotransplantation (involving baboons and genetically engineered pigs), stem cell models (rats, mice and pigs) myocardial infarction with and without reperfusion (rats, mice, rabbits, dogs, and nonhuman primates), hind limb ischemia (rats, mice, rabbits), gene vector delivery to liver (mice, rabbits), and plethysmography (mice). We can also perform cardiac function testing on rats and mice including invasive blood pressure, left ventricular pressure, and pressure-volume loops. Surgical and perioperative support services are provided in the Building 14E Surgical Facility and on the B-2 level of the CRC. We provide support services to all NHLBI animals housed in NIH facilities.

Our ultimate goal is to facilitate getting the research accomplished expeditiously using the best, most humane methods. We will work with each investigator in developing their respective animal model and then either train them in all procedures to enable them to work independently, perform the procedures for them, or work in concert to expedite the animal data generated.

Light Microscopy Core Facility

Christian A. Combs, Ph.D., Facility Director

Building 10, Room 6N-309; E-mail: combsc@nhlbi.nih.gov

Phone: (301) 496-3236; Mobile: 301-768-2568

Daniela A. Malide, M.D., Ph.D., Facility Manager

Building 10, Room 6N-309; E-mail: dmalide@nhlbi.nih.gov

Pager: (301) 402-4719

The mission of the light microscopy core facility is to provide state of the art equipment, training, and image processing capabilities to assist researchers within the NHLBI-DIR in experiments involving light microscopy. Equipment within the facility includes several types of confocal microscopes, a two-photon microscope, and a standard epi-fluorescence widefield microscope. This range of instruments provides capabilities that include live cell imaging, deep tissue-level imaging, video-rate confocal imaging, spectral imaging, and simple widefield fluorescence and brightfield imaging of prepared slides. Image processing capabilities include deconvolution, digital linear unmixing of spectrally overlapping fluorochromes, and 3D reconstruction as well as a custom in-house image processing programs for specific applications. Researchers can work in the facility either collaboratively with Core staff or be trained to work independently on core microscopes and image processing computers.

Murine Phenotyping Core

Danielle Springer, VMD, DACLAM, Director

Building 14E, Room 107A; E-mail: Springerd@nhlbi.nih.gov

Phone: (301) 594-6171; Fax: (301)480-7576

The NHLBI phenotyping core's central mission is development of a comprehensive in-depth knowledge of murine phenotyping methodologies in order to assist investigators with design, research applications, experimental methodologies, data acquisition and interpretation of murine cardiovascular, metabolic, neuromuscular and pulmonary platforms. We seek to provide investigators with high quality scientific and technical support as well as centralized access to state of the art murine phenotyping equipment for the characterization of genetically engineered mouse models. We are currently developing Standard Operating Procedures for our specialized equipment in order to provide high quality, reproducible and reliable data. We provide consultation on appropriate methodologies to acquire cardiovascular, metabolic, neuromuscular, or pulmonary data from your mouse model. The lab assists with experimental design, data collection and acquisition, and data analysis. As most platforms require technical expertise, well developed standard operating procedures, and consistent and refined technique we recommend using our laboratory to collect your data for you. We also are happy to train any interested NHLBI scientist on any SOP, technical skill, equipment operation, etc. that you would like to acquire knowledge on.

NHLBI DirWeb

Mary Anderson, Ph.D., Senior Scientific IT Advisor

Jae Song, Collaborator

Phone: (301) 402-6552; Email: Andersonmk@nhlbi.nih.gov

The NHLBI DirWeb website is available for the principal investigators (PIs) and staff scientists to provide research information and progress reports to the public. The individual laboratories may also use the websites to increase recruitment of both staff scientists and students, and to gain experience and knowledge to broaden the field of expertise and research opportunities. Currently the authorized personnel to manage contents on these websites are the PIs for the group's content, and individual staff scientists for their own staff website. The web management team, led by Dr. Mary Anderson in collaboration with Jae Song, will be available to give technical support to the PIs in constructing the webpage. The web management team is planning to complete this project within NHLBI by June 2010.

Office of Biostatistics Research (OBR)

Nancy L. Geller, Ph.D., Director

Rockledge 2, Room 8210; E-mail: gellern@nhlbi.nih.gov

Phone: (301) 435-0434

The OBR collaborates in the planning, design, implementation, monitoring and analyses of studies funded by NHLBI. OBR also provides statistical consultation to any NHLBI investigator who requests advice and collaborates in data management and analysis of some studies sponsored by the Division of Intramural Research. The professional staff is often asked to serve on in-house administrative committees as well as advisory committees for other Institutes within NIH and other agencies within DHHS. OBR's primary responsibility is to provide objective, statistically sound, and medically relevant solutions to problems that are presented. When a question raised requires new methodology, the OBR is expected to obtain a new and valid statistical solution.

Office of Technology Transfer and Development (OTTAD)

Denise Crooks, Ph.D., Technology Development Specialist

Rockledge 1, Room 6018; E-mail: crooksd@nhlbi.nih.gov

Phone: (301) 435-0103; Fax: (301) 594-3080

OTTAD provides a complete array of services to support the National Heart, Lung, and Blood Institute's technology development activities. To ensure that these activities comply with Federal statutes, regulations and the policies of the National Institutes of Health, a large part of OTTAD's responsibilities includes the day-to-day negotiations of transactional agreements between the NHLBI and outside parties, including universities, pharmaceutical and biotechnology companies. These agreements provide for:

- The exchange of research materials under the Simple Letter of Agreement (SLA) or the Material Transfer Agreement (MTA);
- Collaborative research conducted under cooperative research and development agreements (CRADAs);
- Preclinical and clinical studies of the safety and efficacy of new pharmaceuticals under clinical trial agreements (CTAs); and
- Exchange of confidential information under confidential disclosure agreements (CDAs).

The OTTAD also reviews employee invention reports, generates patentability reports and makes recommendations to the NIH's Office of Technology Transfer (OTT) concerning filing of domestic and foreign patent applications. The OTTAD participates in the marketing of NHLBI technologies as well as provides educational presentations and brochures related to technology transfer for NHLBI scientists. Additionally, the OTTAD advises NHLBI scientists on patent rights, policies, and procedures related to technology transfer. The NHLBI OTTAD staff participates in meetings, discussions and conferences, as appropriate, to stay apprised of and monitor the scientists' needs.

Pathology Core Facility

Zu-Xi Yu, M.D., Ph.D., Facility Head

Building 14E, Room 104A; E-mail: yuz@mail.nih.gov

Phone: (301)496-5035, Fax: (301) 480-6560

The Pathology Core is a Morphology Core Facility, which provides histopathological, immunocytochemical, and ultrastructural support for NHLBI intramural research. The Core Facility provides quality control for morphologic studies, experimental pathology (animal models) and optimizes use of supplies and equipment for all investigators at the NHLBI in which morphological studies and tissue-based molecular studies play a critical role. Services include standard histological and tissue preparation, sectioning and routine staining; frozen tissue section, immunohistochemistry and diagnostic pathology at both histological and ultrastructural level. Ongoing interaction of Pathology Core personnel with each investigator facilitates communication regarding morphologic findings, histopathological interpretation, and new technical developments, thus increasing the efficiency of the research projects. Staff members are well-trained, extremely experienced technicians, and the laboratory has a wide repertoire of specialized techniques. The research pathology and immunohistochemistry are subsequently operating using standard operating procedures based on good lab practice guidelines.

Protein Analysis Core Facility

Duck-Yeon Lee, Ph.D., Director

Building 50, Room 2224 & 2226, E-mail: leedy@nhlbi.nih.gov

Phone: (301) 435-8369, Fax: (301) 451-5459

The mission of the Protein Analysis Core Facility (PAF) is to assist researchers in NHLBI, who want, 1) the accurate mass of protein or peptide and metal content analysis, 2) radioisotope or fluorescence probe labeled protein or peptide isolation, 3) methods development designed to meet the rapidly evolving needs of researchers based on biochemical background. Currently, PAF is operating, 1) ESI-LC/MS, 2) MALDI-TOF, 3) HPLC equipped with the in-line radiochemical or fluorescence detector, 4) Liquid scintillation analyzer, 5) Atomic absorption spectrometer, 6) OFF Gel fractionator, and 7) Bio-Rad open column system. Most instruments are available for "walk-up" use after training by the facility staff.

Proteomics Core Facility

Marjan Gucek, PhD, Director

Building 10, Room 8C103C; E-mail: Marjan.Gucek@nih.gov

Phone: (301) 594-1060

The mission of the NHLBI Proteomics Core Facility is to provide investigators at the NHLBI access to mass spectrometry and gel based proteomics for identification and quantitation of proteins and their posttranslational modifications (PTM). We have state-of-the-art equipment, including latest additions of Orbitrap Velos and 5800 MALDI TOF/TOF. Our workflows for relative protein quantitation are based on DIGE, label-free and iTRAQ approaches. We can also help you identify and quantify protein posttranslational modifications, including phosphorylation, nitrosylation, acetylation, etc. We provide training in proper sample preparation and lead the researchers through mass spectrometric analysis to data searching and interpretation. Users have access to a variety of proteomics software platforms (Sequest, Mascot, Proteome Discoverer, Scaffold, Protein Pilot) for re-searching the data or viewing the results. In addition to helping the NHLBI investigators, we develop new approaches for PTM characterization and absolute protein quantitation.

Transgenic Mouse Core Facility

Chengyu Liu, Ph.D., Director

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Generation of transgenic and knockout mice has remained a challenge for most biomedical laboratories. It requires not only specialized technical skills, but also expensive equipment and critical reagents, such as germline-competent embryonic stem (ES) cell lines. The mission of the NHLBI Transgenic Mouse Facility is to assist NHLBI scientists in creating transgenic and knockout mouse models. Generally, the users of the facility are responsible for making the DNA constructs, and screening the potential positive ES cell clones and founder mice. The staff members at the facility are responsible for culturing, transfecting, and selecting ES cells, as well as microinjecting and implanting mouse embryos. After creation, the transgenic or knockout mice are transferred to each user's animal room for phenotypic analysis. Our standard mouse strain for making transgenic mice is B6CBAF1/J (C57BL/6J x CBA/J), but we can also use FVB/NJ, C57BL/6J, and C57BL6/N inbred strains if necessary. For creating knockout mice, we routinely use embryonic stem (ES) cell lines derived from 129 mouse strain or a hybrid ES cell line derived from 129/C57BL6 F1 hybrid mice. We have also achieved germline transmission using an ES cell line derived from albino C57BL/6 mouse strain, which enables us to generate knockout mice directly on C57BL/6 background. Besides mouse ES cells, our facility is also capable of culturing non-human primate and human ES cells. Recently, we started to explore the methods for generating mouse and human induced pluripotent stem (iPS) cells, as well as the methods for *in vitro* differentiation of ES and iPS cells.

Poster Session Titles and Assignments

Session 1: Wednesday from 4:30-7:00pmAuthors of odd posters present 4:30 to 5:45Authors of even present 5:45 to 7:00**Session 2: Thursday from 4:00-6:30pm**Authors of odd posters present 4:00 to 5:15Authors of even posters present 5:15 to 6:30

History of Baltimore

Baltimore was founded in 1729 and is the largest city in the state of Maryland. Baltimore's Inner Harbor was once the second leading port of entry for immigrants to the United States and major manufacturing center. Baltimore played a key part in events leading to and including the American Revolution. On September 13, 1814 the British attacked Baltimore but Fort McHenry forces successfully defended the city's harbor. Francis Scott Key witnessed this attack and later wrote "The Star-Spangled Banner," which are the words to the United States National Anthem.



In 1904 a fire destroyed over 1,500 buildings and most of the city had to be rebuilt. During the 1970's, Baltimore's downtown area known as the Inner Harbor was occupied by a collection of abandoned warehouses. Efforts to redevelop the downtown area started with the construction of the Baltimore Convention Center in 1979. One year later, retail and restaurants began to surface. The National Aquarium was also built to attract tourism. In 1992, the Baltimore Orioles baseball team moved to Camden yards near the harbor. The Baltimore Ravens football team moved to M&T Bank Stadium next to Camden Yards in 1998.

Baltimore is historically known as a working-class port town. Most notable today are three downtown areas along the port: the Inner Harbor, frequented by tourists due to its hotels, shops, and museums; Fells Point, once a favorite entertainment spot for sailors but now refurbished and gentrified; and Little Italy located between the other two, where Baltimore's Italian-American community is based. Further inland, Mt Vernon, is the traditional center of cultural and artistic life of the city; it is home to a distinctive Washington Monument, set atop a hill in a 19th century urban square, that predates the more well-known monument in Washington, D.C. by several decades.

Today, Baltimore is a thriving city home to fortune 500 companies. It offers many tourist attractions including the Maryland Science Center, Edgar Allen Poe House and Museum, and the National Great Blacks in Wax Museum.



Photos courtesy of baltimore.org
History of Baltimore provided by wikipedia.org

Wednesday**Biochemistry/Biophysics**

1. **A Computational Investigation of the Nitrogen-Boron Interaction in o-(N,N-Dialkylaminomethyl)arylboronate Systems.** J. D. Larkin, J. S. Fossey, T. D. James, C. W. Bock, and B. R. Brooks, Laboratory of Computational Biology/Computational Biophysics Section
2. **A non-proteasomal catabolic pathway for oxidized protein – IRP2 as a model system.** A. H. K. Chang, J. Jeong, R. L. Levine, Biochemistry and Biophysics Center
3. **Characterization of the Interaction Between BetaPix and PDZ Domain of Sorting Nexin 27 in Kidney Epithelial Lysate** J. L. Valdes, M. P. Playford, S. L. Milgram, Laboratory of Epithelial Cell Biology
4. **Copper(I) and Copper(II) Binding to a-Synuclein.** H. R. Lucas and J. C. Lee, Laboratory of Molecular Biophysics
5. **Expression of SR-B1 in the Endothelium Enhances Reverse Cholesterol Transport.** M. Ghias, B. Vaisman, A. Remaley, Pulmonary and Vascular Medicine Branch
6. **How do the co-repressor HDACs act in the human genome.** Z. Wang¹, C. Zang², K. Cui¹, W. Peng², and K. Zhao¹, ¹Laboratory of Molecular Immunology, ²Physics Dept., George Washington University
7. **Investigating the single molecule kinetics of myosin 7a using an optical trap.** A. Roka¹, V.B. Siththanandan¹, Y. Takagi¹, Y. Yang¹, D.K.T. Hong², J.R. Sellers¹, ¹Laboratory of Molecular Physiology, ²Summit Computers, Washington DC
8. **MAT1, An Acetyltransferase Conserved From Prokaryotes, Regulates Mammalian Mitochondrial Respiration.** I. Scott, B. R. Webster, M. N. Sack, Translational Medicine Branch
9. **Membrane diffusion of tethered DPPC and tethered PIP3-bound protein systems.** M. G. Lerner and R. W. Pastor, Laboratory of Computational Biology
10. **Methionine Sulfoxide Reductase A Overexpression in Murine Embryonic Fibroblast and Hepatocyte does not Improve Cellular Resistance Against Oxidative Stress.** H. Zhao, G. Kim, C. Liu, R. L. Levine, Lab of Biochemistry
11. **Mitochondrial Acetyltransferase I (MAT1) may function as a ‘nutrient sensor’ regulating autophagy.** B. R. Webster, I. Scott, M. V. Stevens, M. N. Sack, Translational Medicine Branch
12. **STAT3 and Mitochondrial Function in the Hyperimmunoglobulin E Syndrome.** R. Huang, K. Kim, C. Avila, A. Freeman, A. Aponte, M. Boehm, S. Holland, J. Milner, M Sack, Translational Medicine Branch.
13. **Mitochondrial respiration Protects Against Oxygen-associated DNA Damage.** H. J. Sung, W. Ma, P. Wang, J. Hynes¹, T. C. O’Riordan¹, C. A. Combs², P. J. McCoy³, F. Bunz⁴, J. Kang and P. M. Hwang, Translational Medicine Branch, ¹ Luxcel Biosciences Ltd., Ireland, ² Light Microscopy Core Facility, ³ Flow Cytometry Core Facility, ⁴ Radiation Oncology Department, Johns Hopkins University School of Medicine
14. **Multi-Scale Modeling of Coarse Grained Protein Interactions: A CHARMMing Implementation.** F. C. Pickard IV, B. T. Miller, H. L. Woodcock, H. F. Schaefer III, B. R. Brooks, Laboratory of Computational Biology

15. **Pink1 Preserves Cardiac Function in Response to Pressure-Overload and Aging Induced Stress Through Regulating Mitochondrial Dynamics.** M. V. Stevens, K. Y. Kim, D. Springer, S. Andersen, A. Noguchi, S. Esfahani, M. Daniels, H. San, M. N. Sack, Translational Medicine Branch
16. **Single Molecule Investigation Of The Acto-Myosin-10 Complex.** N. Billington¹, Y. Takagi¹, R. E. Farrow², S. Guzik¹, G. I. Mashanov², Y. Yang¹, E. E. Homsher³, D. K.T. Hong⁴, J. E. Molloy² and J. R. Sellers¹,¹Lab of Molecular Physiology, NHLBI, National Institutes of Health, Bethesda, MD,²MRC National Institute for Medical Research, London, U.K.,³David Geffen School of Medicine at UCLA, Los Angeles, CA, ⁴Summit Computers, Washington, DC
17. **Single- Molecule Study of Human Topoisomerase II.** Y. Seol¹, A. C. Gentry², N. Osheroff², and K. C. Neuman¹,¹National Institutes of Health, Bethesda, ²Vanderbilt University, Nashville, TN
18. **Site Specific Fluorescent Probes of α -Synuclein Fibril Assembly.** T. L. Yap, C. M. Pfefferkorn, and J. C. Lee, Laboratory of Molecular Biophysics

Genetics

19. **Alterations in Cardiac microRNA Expression Between Male and Female Mice.** A. M. Deschamps¹, R. Wang², D. Liu², N. Raghavachari², E. Murphy¹, ¹ Translational Medicine Branch and ²Microarray Core
20. **Characterization of Zebrafish Glycoproteins 7a and 7b as Heparan Sulfate Proteoglycans, and Specifically as Glypicans.** C. D. Liepmann, K. Kramer, Genetics and Development Biology Center
21. **Computational Prediction of *Drosophila* Muscle Enhancers.** B. W. Busser¹, L. Taher², A. A. Philippakis³, M. L. Bulyk³, I. Ovcharenko² and A. M. Michelson¹, ¹Laboratory of Developmental Systems Biology, ²Computational Biology Branch, National Center for Biotechnology Information, and ³Division of Genetics, Department of Medicine, Brigham and Women's Hospital, Boston, MA
22. **Glypicans Coordinately Regulate Zebrafish Dorsoventral Development.** C. Tharp, A. Srinivasan, K. L. Kramer; Developmental Glycobiology Section
23. **Landscape of Histone Variant H2A.Z in Mouse Embryonic Stem Cells.** G. Q. Hu, K. Cui, Q. Tang, C. Crane-Robinson, K Zhao, Laboratory of Molecular Immunology
24. **Modeling the genetic basis for human congenital heart disease using forward genetic screening with mouse ENU mutagenesis.** B. Chatterjee, Q. Yu, Y. Shen, S. Sabol, L. Leatherbury, C.W. Lo, Laboratory of Developmental Biology
25. **Multiple Platelet Aggregation Genes are Identified by Genome-wide Association Meta-analyses.** A. D. Johnson, L. R. Yanek, M. H. Chen, A. T. Kraja, M. G. Larson, R. Lin, G. H. Tofler, D.M. Becker, Q. Yang, M. A. Province, C. J. O'Donnell, L. C. Becker, Center for Cardiovascular Genomics, The Framingham Heart Study
26. **Pol II and its associated epigenetic marks are present at pol III-transcribed non-coding RNA genes.** A. Barski¹, I. Chepelev¹, D.Liko², S. Cuddapah¹, A. B. Fleming², J. Birch², K. Cui¹, R. J. White² and K. Zhao¹, ¹Laboratory of Molecular Immunology, ²Beatson Institute for Cancer Research, Gartcube Estate, Switchback Road, Bearsden, Glasgow, UK

Injury/Inflammation/Immunology

27. **Actomyosin Contraction Drives TCR Microcluster Movement at the pSMAC and Formation of cSMAC at the IS in T cells.** C. Yi, X. Wu, J. Hammer, Laboratory of Cell Biology
28. **An Apolipoprotein E Mimetic Peptide Inhibits Airway Hyperreactivity in a House Dust Mite Model of Allergic Asthma.** X. L. Yao, K. Fredriksson, Z. X. Yu, X. L. Xu, N. Raghavachari, K. J. Keeran, G. J. Zwicke, M. J.A. Amar, A. T. Remaley, and S. J. Levine, Pulmonary and Vascular Medicine Branch, Pathology Core Facility, Gene Expression Core Facility, and Laboratory of Animal Medicine and Surgery
29. **Exosomes derived from antigen-pulsed immature dendritic cells attenuate airway inflammation and hyperreactivity in a murine model of allergic asthma.** K. Fredriksson, X-L. Yao, J. K. Lam, H. B. Bhakta, S. J. Levine, Pulmonary and Vascular Medicine Branch
30. **Not All C57BL/6 Mice Are Created Equal.** M. Bourdi, J. S. Davies, K. Sendide, and L .R. Pohl, Laboratory of Molecular Immunology
31. **STAT3 and Mitochondrial Function in the Hyperimmunoglobulin E Syndrome.** R. Huang, K. Kim, C. Avila, A. Freeman, A. Aponte, M. Boehm, S. Holland, J. Milner, M Sack, Translational Medicine Branch
32. **Sunitinib Selectively Attenuates Airway Hyperreactivity, but not Airway Inflammation, in a Murine Model of Allergic Asthma.** J. K. Lam¹, H. C. Bhakta¹, J. Zhang¹, K. Fredriksson¹, M. Yu¹, X. Yao¹, K. J. Keeran², G. J. Zywicke², Z. X. Yu³, S. J. Levine¹, ¹Pulmonary and Vascular Medicine Branch, ²Laboratory of Animal Medicine and Surgery, and ³Pathology Core Facility

Physiology

33. **Ca²⁺ sensing receptor (CaSR): a mediator of ischemic preconditioning in the hearts?** J. Sun, E. Murphy, Translational Medicine Branch
34. **Estrogen reduces ischemia-reperfusion (I/R) injury via PI3-kinase and phosphorylation of mitochondrial dehydrogenases.** C. Lagranha¹, A. Deschamps¹, A. Aponte¹, C. Steenbergen², E. Murphy¹, Translational Medicine Branch¹, Johns Hopkins University²
35. **Identifying Components of the Mitochondrial Permeability Transition Pore and Its Physiological Role Through Cyclophilin D Interactions.** R. Wong, G. Wang, M. Gucek, J. D. Molkentin, C. Steenbergen, E. Murphy, Translational Medicine Branch
36. **Large Scale Profiling of Protein Degradation Rates and Translation Rates In Renal Collecting Duct Cells.** D. H. Slentz, M. J. Yu, T. Pisitkun, J. D. Hoffert, M. A. Knepper. Epithelial Systems Biology Laboratory
37. **Phospho-specific Antibodies to Ser126 and Ser874 of NKCC2 Demonstrate Vasopressin Regulated Phosphorylation in Renal TAL.** R. Gunaratne, T. Pisitkun, D.W.W. Braucht, L. Xie, M.M. Rinschen, J.D. Hoffert, C.L. Chou, M.A. Knepper, Epithelial Systems Biology Laboratory
38. **Potential Roles of PDE3B Knockout in Acquisition of Brown Fat Characteristic by White Adipose Tissue in Mice.** E. Zmuda-Trzebiatowska, V. Manganiello, Translational Medicine Branch
39. **S-nitrosylation Exerts Cardioprotection During Ischemia-Reperfusion Injury by Reducing Cysteine Oxidation.** M. J. Kohr^{1,2}, J. Sun¹, C. Steenbergen², E. Murphy¹, ¹Translational Medicine Branch, ²Department of Pathology, Johns Hopkins University, Baltimore, MD

Techniques/Imaging

40. **Adeno-associated virus-2 (AVV2) integrase mediates specific integration to locus AAVS1 on chromosome 19q13.3.** A. E. Dunfee, J. R. Clevenger, C. E. Dunbar, and A. Larochele, Hematology Branch
41. **A New synthetic route to CCF2/AM: An Imaging Agent for the Detection of Single Cell Protease Activity *in situ* and Pharmacological Inhibition Studies.** H. Wu, Z-D. Shi, C. Li, Y. Hu, G. L. Griffiths, B. M. Connolly and T. H. Bugge. The Imaging Probe Development Center at NHLBI in collaboration with NIDCR
42. **Characterization of Chondroitin Sulfate Using High Resolution Ion Trap Time-of-Flight Mass Spectrometry** Y. Katagiri, Y. Wang*, P. Yu, J. H. Yi, D. A. Figge, F. Hays* H. M. Geller, Developmental Neurobiology Group, *Shimadzu Scientific Instruments
43. **Clinical Development of Panitumumab-CHX-A”DTPA as a Potential SPECT Diagnostic Imaging Agent.** S. Cheal, O. Vasalatiy, S. Cofiell, A. Bate, J. L. Tatum, G. L. Griffiths, The Imaging Probe Development Center at NHLBI in collaboration with NCI
44. **Combining Conformational Space Annealing with Replica Exchange Method for Improved Conformational Search.** A. Okur, J. Lee, B. Brooks, Laboratory of Computational Biology
45. **Constant pH Molecular Dynamics Simulations with the Replica-Exchange Method in the pH space.** S. G. Itoh, A. Damjanovi□, B. R. Brooks, Laboratory of Computational Biology
46. **Gold Nanoparticles as potential cancer imaging and therapeutic agents.** A. Sulima, B. Xu, N. Shenoy, G. L. Griffiths, J. Capala, G. Kramer-Marek, The Imaging Probe Development Center at NHLBI in collaboration with NCI
47. **Improving the Stability, Capability and Resolution of the Dual-Beam Optical Trap *in vitro* Force Assay.** S. Hernandez¹, Y. Takagi², E. E. Homsher^{2,3} and J. R. Sellers^{2, 1} Stony Brook University, Stony Brook, NY, ²Laboratory of Molecular Physiology, ³David Geffen School of Medicine at UCLA, Los Angeles, CA
48. **Maximizing Signals from *in vivo* Multiphoton Microscopy: Non-contact Total Emission Detection (epi-TED).** A. V. Smirnov¹, C. A. Combs², David Chess³, M. Luger-Hamer³, D. B. McGavern⁴, S. S. Kang⁴, J. R. Knutson¹, and R. S. Balaban³, ¹Laboratory of Molecular Biophysics, ²Light Microscopy Facility, ³Laboratory of Cardiac Energetics, ⁴NINDS Viral Immunology and Intravital Imaging Unit
49. **Motion Tracking Using Optical Navigation During *in-vivo* Two-Photon Microscopy.** J. Schroeder¹, R. Pursley², M. Bakalar², T. Pohida², P. Kellman¹, R. Balaban¹, ¹Laboratory of Cardiac Energetics, NHLBI, ²Signal Processing and Instrumentation Section, CIT
50. **MSCALE: A Framework for Multiscale Molecular Simulations in CHARMM.** B. T. Miller, H. L. Woodcock, M. Hodoscek, A. Okur, J. D. Larkin, and B. R. Brooks, Laboratory of Computational Biology
51. **Production and Preliminary Testing of Ultra-Stable Gadolinium-Benzyl-DOTA-Cholera Toxin B Conjugates as MRI Brain Circuitry Tracking Agents.** O. Vasalatiy, S. Cheal, C. W. H. Wu, R. B. Tootell, A. P. Koretsky, L. Ungerleider, G. L. Griffiths, The Imaging Probe Development Center at NHLBI in collaboration with NINDS &NIMH

52. **Simultaneous Myocardial Perfusion and Strain Imaging with Displacement- encoded MRI.** Y. Le, K. Peter, J. Taylor, E. Bennett, K. Lucas, C. Ched'Hotel, C. H. Lorenz, P. Croisille, H. Wen, Translational Medicine Branch
53. **Synthesis of ⁶⁸Ga-Radiolabeled Proteins and Peptides for Positron Emission Tomography.** N. Shenoy, J. Capala, G. Kramer, G. L. Griffiths, Imaging Probe Development Center at NHLBI and NCI
54. **Synthesis of ApoSense compound [¹⁸F] 2-(5-(dimethylamino)naphthalene-1-sulfonamido)-2-(fluoromethyl)butanoic acid (NST732) via nucleophilic ring-opening of activated aziridine.** F. Basuli, H. Wu, B. Teng, Z-D Shi, Y. Hu, J.L. Tatum, G. L. Griffiths, Imaging Probe Development Center
55. **Synthesis of UDP-2-Ketogalactose and UDP-2-Ketoglucose for the Site-Specific Linkage of Biomolecules via Modified Glycan Residues using Glycosyltransferases.** A. E. Dulcey, J. Lamb, P. K. Qasba, G.L. Griffiths, The Imaging Probe Development Center at NHLBI in collaboration with NCI
56. **Three Dimensional Superresolution Fluorescence Microscopy Reveals Protein Stratification in Focal Adhesions.** P. Kanchanawong, G. Shtengel, A. M. Pasapera, Ericka B. Ramko, M. W. Davidson, H. F. Hess, C. M. Waterman, Laboratory of Cell and Tissue Morphodynamics
57. **Visualization Of Dynamic Active Devices Via Adaptive Undersampled Projection Imaging In MRI-guided Interventional Procedures.** A. K. George, C. E. Saikus, O. Kocaturk, R. J. Lederman, A. Z. Faranesh, Translational Medicine Branch

Thursday

Cell Biology

1. **An RNAi Screen of Microtubule-Regulatory Proteins Identifies MARK2/Par1 as an Effector of Rac1-mediated Microtubule Growth.** Y. Nishimura¹, K. Applegate², G. Danuser³, C. Waterman¹; ¹ Laboratory of Cell and Tissue Morphogenesis, ² Laboratory for Computational Cell Biology, The Scripps Research Institute, La Jolla, CA, ³ Laboratory for Computational Cell Biology, Harvard Medical School, Boston, MA
2. **Characterization of integrin “puffs”: a dynamic subset of ECM-bound integrins in adherent tissue cells that are not associated with focal adhesions.** L. B. Case and C. M. Waterman, Laboratory of Cell and Tissue Morphogenesis
3. **Characterization of the Interaction between Zona Occludens-2 and Sorting Nexin 27 in Kidney Epithelial Cells.** S. Zimmerman, A. Udofa, M. Playford, S. Milgram Laboratory of Kidney and Electrolyte Metabolism
4. **Distinct Sorting Determinants Guide the Trafficking Itinerary of the New Clathrin-independent Endocytic Cargo Proteins CD44 and CD147.** L. Maldonado-Báez, N. B. Cole, C. A. Eyster and J. G. Donaldson, Laboratory of Cell Biology
5. **Extracellular Matrix Remodeling and Hif-1a Signaling: New Insights From A Patient With Prolidase Deficiency.** A. D. Walts, C. St. Hilaire, D. Kastner, I. Aksentijevich, M. Boehm, Translational Medicine Branch
6. **Fluorescent Probes of Membrane-bound Alpha-synuclein: Insights into the Role of Membranes in Aggregation.** C. M. Pfefferkorn and J. C. Lee, Laboratory of Molecular Biophysics
7. **Kinetic Characterization of Non-muscle Myosin IIB SH-HMM and HMM on Single Molecule Level with Optical Tweezers.** A. Nagy¹, Y. Takagi¹, E. Homsher^{1,2}, D. K. T. Hong³, M.

Kovács⁴ and J. R. Sellers¹,¹Laboratory of Molecular Physiology, ²Department of Physiology, David Geffen School of Medicine at UCLA, ³ABT Software, Washington, D.C., ⁴Department of Biochemistry, Eötvös University

8. **MARCH Proteins Promote Delivery of Clathrin-Independent Endocytic Cargo Proteins MHC1 and CD98 to Late Endosomes.** C. Eyster, K. Viswanathan, K. Früh, and J. Donaldson, Laboratory of Cell Biology
9. **Myosin II activity regulates vinculin recruitment to focal adhesions through FAK-mediated paxillin phosphorylation.** A. M. Pasapera¹, I. C. Schneider³, E. Rericha², D. D. Schlaepfer⁴, C. M. Waterman¹, ¹Cell Biology and Physiology Center, ²Institute for Research in Electronics and Applied Physics, University of Maryland, College Park, MD, ³Departments of Chemical and Biological Engineering; Genetics, Development and Cell, Biology, Iowa State University, Ames, IA, ⁴Moore's UCSD Cancer Center and Department of Reproductive Medicine, University of California, San Diego, La Jolla, CA
10. **Myosin-II Mediated Regulation of Microtubule Dynamics Controls Endothelial Cell Branching Morphogenesis.** K. A. Myers¹, K. T. Applegate², R. S. Fischer¹, G. Danuser³, C. M. Waterman¹, ¹Laboratory of Cell and Tissue Morphodynamics, ²Laboratory for Computational Cell Biology, The Scripps Research Institute La Jolla, CA, ³Laboratory for Computational Cell Biology, Harvard Medical School, Boston, MA
11. **Paxillin Phosphorylation Is a Local Regulator of Mechanotransduction Within Individual Focal Adhesions.** S. V. Plotnikov¹, B. Sabass², A. Pasapera¹, U. S. Schwarz², C. M. Waterman¹, ¹Laboratory of Cell and Tissue Morphodynamics, ²University of Heidelberg, Heidelberg, Germany
12. **Proteomic Analysis of Myosin II-mediated Focal Adhesion Maturation.** J. Kuo¹, X. Han², J. Yates², C. M. Waterman¹, ¹Cell Biology and Physiology Center, ²Cell Biology, Scripps Research Institute, La Jolla CA
13. **The Calcium Channel MCOLN3 Regulates Endosomal Acidification.** B. Lelouvier and R. Puertollano. Laboratory of Cell Biology
14. **Vinculin stabilizes nascent adhesions and establishes a lamellipodium-lamella border in migrating cells.** I. Thievessen, S. Berlemont, A. Zemljic-Harpf, R. S. Ross, C. M. Waterman, Laboratory for Cell and Tissue Morphodynamics

Cell Cycle/Cancer

15. **BCL2A1 is a Survival and Immortalization Factor for Primitive Hematopoietic Cells.** J. Y. Métais, T. Winkler, R. T. Calado, and C. E. Dunbar. Hematology Branch
16. **Inhibition of Lipid Synthesis Activates the DNA Damage Response and Triggers Premature Senescence in Human Fibroblasts.** C. Quijano, L. Cao, J. Liu, M. Fergusson and T. Finkel, Translational Medicine Branch
17. **Polo-Like Kinases Mediate Cell Survival In Mitochondrial Dysfunction.** P. Wang, T. Matsumoto, W. Ma, H.J. Sung, S. Matoba, and P. M. Hwang, Translational Medicine Branch
18. **The essential autophagy gene Atg7 coordinates cell cycle withdrawal and survival with nutrient status.** I. H Lee, L. Cao, Y. Kawai, M. M. Fergusson, I. Rovira, A. J.R. Bishop, N. Motoyama and T. Finkel, Molecular Biology Section
19. **The role of testosterone replacement therapy on telomere preservation in normal and telomerase-deficient mice.** M. J. Bachman, M. J. Desierto, J. Chen and N. S. Young

20. **DNA damage responses in BRCA1 deficiency induced apoptosis, senescence and tumorigenesis.** L. Cao^{1,2}, C. X. Deng² and T. Finkel¹, ¹Translational Medicine Research Branch, NHLBI, ²Genetics of Development and Diseases Branch, NIDDK

Clinical Studies

21. **Endothelial Cell Activation Induced by Plasma from Patients with Sickle Cell Disease.** A. Ask, L. Mendelsohn, X. Wang, G. J. Kato, Sickle Cell Vascular Disease Group
22. **Telomere Shortening Promotes Chromosomal Instability and Predicts Malignant Clonal Evolution in Aplastic Anemia.** R. T. Calado¹, J. N. Cooper¹, P. Scheinberg¹, C. Wu¹, M. A Zago², H. Padilla-Nash³, T. Ried³, E. M Sloand¹ and N. S. Young¹, ¹Hematology Branch, ²Department of Internal Medicine, University of Sao Paulo at Ribeirao Preto Medical School, Ribeirao Preto, Brazil, ³Genetics Branch
23. **Epidemiology of Vaso-occlusive Pain in Sickle Cell Anemia and Its Association with a Susceptibility Marker in the *GCH1* Gene.** D. Darbari, I. Belfer, V. Youngblood, K. Desai, L. Diaw, L. Freeman, M. Hildeshem, C. Minniti, V. Nolan, J. N. Milton, S.W. Hartley, M. H. Steinberg, D. Goldman, M.B. Max, G. Kato, J. G. Taylor VI, Sickle Cell Disease Vascular Disease Unit, Pulmonary and Vascular Medicine Branch
24. **Fatty Kidney is Associated with Hypertension and Chronic Kidney Disease: the Framingham Heart Study.** M. C. Foster, S-J Hwang, J. M. Massaro, U. Hoffmann, C. S. Fox, Center for Population Sciences, Framingham Heart Study
25. ***FOS* Expression in Blood as a LDL-Independent Marker of Statin Treatment.** J. G. Kang, H. J. Sung, S. I. Jawed, C.L. Brennen, Y. N. Rao, S. Sher, L. G. Biesecker, A. A. Quyyumi, V. Sachdev, P. M. Hwang, Translational Medicine Branch
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Jumeau is Essential for Proper Symmetric and Asymmetric Cell Division During *Drosophila* Cardiogenesis. S. M. Ahmad, A. M. Michelson, Laboratory of Developmental Systems Biology

The development of a complex organ comprising multiple cell types requires (i) the proper differentiation of these cell types, (ii) the production of appropriate numbers of cells for each cell type, and (iii) the correct positioning of these cells with respect to one another in the organ. We show that, in the formation of the *Drosophila* heart, all three of these processes are mediated by *jumeau* (*jumu*), a gene which is expressed in the cardiac mesoderm and which encodes a forkhead-family transcription factor. Embryos lacking *jumu* function exhibit localized changes, both increase and reduction, in cardioblast number as well as misaligned and incorrectly positioned heart cells. We demonstrate that these phenotypes are a consequence both of (i) *jumu* playing an integral role in asymmetric cell division at two different embryonic stages to bring about the proper derivation of two distinct types of heart cells, *Seven-up* expressing myocardial cells and *Seven-up* expressing pericardial cells, from their mutual precursor; and of (ii) *jumu* also being involved in the symmetric cell division that gives rise to yet another cardiac cell type, the *tinman*-expressing myocardial cells. We are examining the possibility that *jumu*'s roles in both asymmetric and symmetric cell division are mediated by *polo*, a kinase-encoding gene which we also found to be expressed in the cardiac mesoderm, which exhibited a loss-of-function phenotype similar to that of *jumu*, and which is known to be essential during asymmetric cell division for the proper localization of Numb protein, a process which goes awry in *jumu* mutants.

Endothelial Cell Activation Induced by Plasma from Patients with Sickle Cell Disease. A. Ask, L. Mendelsohn, X. Wang, G. J. Kato, Sickle Cell Vascular Disease Group

Sickle Cell Disease (SCD) is a genetic blood disorder causing deformation of red blood cells under hypoxic conditions with the consequence of organ ischemia and hemolysis. Our group has shown that intravascular hemolysis results in low nitric oxide bioavailability and endothelial activation, giving rise to vasoconstriction, platelet activation, vasculopathy, and possibly promoting pulmonary hypertension. Adhesion molecules are involved in blood cell interaction with the vascular endothelium, and soluble components of these receptors have been reported by our lab and others at increased levels in SCD plasma, correlated with pulmonary hypertension.

Here, we investigate the effect of SCD plasma on cultured vascular endothelium to test the hypothesis that certain plasma factors contribute to endothelial activation and dysfunction, possibly related to pulmonary hypertension. A cell-based enzyme-linked immunosorbent assay has been established to detect enhanced adhesion molecule expression (ICAM, VCAM, P-selectin, CD40) on the endothelial cell surface in response to exposure to human plasma, indicating endothelial activation. TNF-alpha is a known inducer of adhesion molecules and lead to a 5-fold increase of ICAM, but only to an up to 1.5-fold increase of VCAM, P-selectin and CD40. Preliminary results show that SCD plasma also induces adhesion molecule expression, in one case leading to a 3-fold up-regulation. We will report on the results from this assay on a larger number of SCD samples to

assess whether adhesion molecule expression correlates with clinical parameters.

The Role of Testosterone Replacement Therapy on Telomere Preservation in Normal and Telomerase-deficient Mice. M. J. Bachman, M. J. Desierto, J. Chen and N. S. Young Hematology Branch

Telomere shortening causes DNA instability contributing to tumorigenesis, cell senescence, and the development of a broad range of diseases including bone marrow (BM) failure syndromes. It has been shown that androgen therapy can restore cellular telomerase activity in vitro and can improve blood cell counts in vivo in dyskeratosis congenital and aplastic anemia patients with telomerase mutations. In the current study, we tested the effectiveness of testosterone therapy in the preservation of telomere length in vivo in C57BL/6 (B6) and telomerase RNA component deficient (*Terc*^{-/-}) mice with or without orchiectomy surgery. Mice received subcutaneous injection of 250 g/Kg testosterone once every week, or equal volume of corn oil as controls. All animals were bled before and nine weeks after the beginning of the treatment to extract DNA for the measurement of relative telomere length using a qPCR method. Data from our current study showed that orchiectomy accelerated telomere attrition in both B6 and *Terc*^{-/-} mice. Testosterone treatment preserved telomere length in general, with the effect being significant in B6 mice ($P < 0.05$) but not in *Terc*^{-/-} mice. In B6 animals, relative telomere length shortened in orchiectomized mice with corn oil (-0.1515 ± 0.0609), non-orchiectomized mice with corn oil (-0.0189 ± 0.0609), and non-orchiectomized mice with testosterone (-0.0867 ± 0.0862), but telomere length extended significantly ($P < 0.05$) in orchiectomized mice with testosterone treatment (0.1061 ± 0.0862). Thus, testosterone replacement therapy modulated telomerase activity and preserved, or even extended, telomere length.

Pol II and its Associated Epigenetic Marks Are Present at Pol III-transcribed Non-coding RNA Genes. A. Barski¹, I. Chepelev¹, D. Liko², S. Cuddapah¹, A. B. Fleming², J. Birch², K. Cui¹, R. J. White² and K. Zhao¹, ¹Laboratory of Molecular Immunology, ²Beatson Institute for Cancer Research, Garscube Estate, Switchback Road, Bearsden, Glasgow, UK

Epigenetic control is an important aspect of gene regulation. Despite detailed understanding of protein-coding gene expression, the transcription of non-coding RNA genes by RNA polymerase (pol) III is less well characterized. Here we profile the epigenetic features of pol III target genes throughout the human genome. This reveals that the chromatin landscape of pol III-transcribed genes resembles that of pol II templates in many ways, although there are also clear differences. Our analysis also discovered an entirely unexpected phenomenon, namely that pol II is present at the majority of genomic loci that are bound by pol III.

Synthesis of Aposense Compound [¹⁸F] 2-(5-(Dimethylamino)naphthalene-1-sulfonamido)-2-(Fluoromethyl)butanoic Acid (Nst732) via Nucleophilic Ring-opening of Activated Aziridine. F. Basuli, H. Wu, B. Teng, Z-D Shi, Y. Hu, J.L. Tatum, G. L. Griffiths, Imaging Probe Development Center

The Imaging Probe Development Center at NHLBI in collaboration with NCI 2-(5-(dimethylamino)naphthalene-1-

sulfonamido)-2-(fluoromethyl)butanoic acid (NST732) is a member of the ApoSense® family of compounds, capable of selective targeting, binding and accumulation within cells undergoing apoptotic cell death. It has application in molecular imaging and blood clotting particularly for monitoring anti-apoptotic drug treatments and we are investigating a fluorine-18-radiolabeled analog for positron emission tomography. For its radiosynthesis, reaction of the tosylate precursor methyl 2-(5-(dimethylamino)naphthalene-1-sulfonamido)-2-(tosyloxymethyl)butanoate in 1:1 acetonitrile, dimethylsulfoxide with [¹⁸F] tetrabutyl ammonium fluoride (TBAF) proceeds through an aziridine intermediate to afford two regioisomers [¹⁸F] 2-(5-(dimethylamino)naphthalene-1-sulfonamido)-2-fluorobutanoate and [¹⁸F] methyl 2-(5-(dimethylamino)naphthalene-1-sulfonamido)-2-(fluoromethyl)butanoate. [¹⁸F] 2-((5-Dimethylamino)naphthalene-1-sulfonamido)methyl)-2-fluorobutanoic acid (NST732) was then obtained by the hydrolysis of corresponding [¹⁸F]-labeled ester with 6N hydrochloric acid. The total radiochemical yield was 7% (uncorrected) in a 90 minute synthesis time. In conclusion, we have established a convenient synthetic pathway to prepare both [¹⁸F]-radiolabeled NST732 and its corresponding reference standard compounds.

Identifying Novel Binding Partners of Glypicans and Syndecans. L. Belland, Y. Kee, A. Srinivasan, K. Kramer, Laboratory of Developmental Biology

Heparan sulfate proteoglycans (HSPGs), which are composed of heparan sulfate chains attached to core proteins, mediate signaling events in development, homeostasis and disease by interacting with major cell-signaling molecules. Glypicans and syndecans, the main cell-surface HSPGs, have been shown to bind several extracellular molecules in a heparan sulfate (HS)-dependent manner. It was recently reported, however, that the HS chains may not be required for some interactions between glypicans and their ligands, indicating that their core proteins may directly bind some signaling molecules. Therefore, to better understand the roles of HSPGs in regulating signaling, the purpose of our project is two-fold: to determine novel interactions between glypicans and major signaling molecules, and to examine whether glypican and syndecan core proteins have their own binding specificity to these binding partners. To address these questions, we have adopted two different systems to detect the protein-protein interactions, one for HS-dependent binding interactions and the other for HS-independent binding interactions. For the former, we are developing an alkaline phosphatase assay and immunoprecipitation assay, which we will use to detect interactions between the six human glypicans and various signaling molecules. For the latter, we have set up a yeast two-hybrid system which we have used to test the ability of glypican and syndecan core proteins to form homo- and heterodimers. Preliminary data indicate that there may be some glypican-glypican and glypican-syndecan core protein heterodimer interactions. These results implicate that core protein-protein interactions may contribute to an additional level of regulation and fine tuning in signaling events.

Single Molecule Investigation of the Acto-myosin-10 Complex. N. Billington¹, Y. Takagi¹, R. E. Farrow², S. Guzik¹, G. I. Mashanov², Y. Yang¹, E. E. Homsher³, D. K.T. Hong⁴, J. E. Molloy² and J. R. Sellers¹, ¹Lab of Molecular Physiology, NHLBI, National Institutes of Health, Bethesda, MD, ²MRC National Institute for Medical Research, London, U.K., ³David Geffen

School of Medicine at UCLA, Los Angeles, CA, ⁴Summit Computers, Washington, DC

Recent cell biological studies of myosin-10, an actin-stimulated ATPase molecular motor, have revealed that it is essential to cellular processes such as filopodia extensions and phagocytosis. Steady-state and transient kinetic biochemical studies of the ATPase cycle of a single-headed, subfragment-1 like (S1), construct show that it has an intermediate duty-cycle ratio – *i.e.* it remains tightly bound to actin for about 16% of its total ATPase cycle time but spends around 90% associated with actin in both weak and strongly bound states. Furthermore, the acto-myosin-10-S1 complex has two ADP bound states, as well as a surprisingly high affinity for actin, comparable to that of the rigor complex between actin and skeletal muscle myosin II. For this study we used a two-headed, heavy meromyosin-like (HMM) construct of myosin-10, containing a leucine-zipper at the C-terminal end to force dimerization of the protein. We used negative stain electron microscopy and single particle image processing. Images confirmed the dimerization of the construct and allowed measurement of the contour length of dimerized molecules. Contour length measured from the tip of one motor to the tip of the second motor was 50.6nm ± 5.5nm SD. Image processing of the myosin bound to actin showed a similar structure of the bound motor domain to that seen previously with a range of myosins but a lever which tends to be seen at a shallower angle with respect to the actin filament than has been seen previously in similar studies of myosins bound to actin, such as myosin-5 and myosin-2 from muscle. In order to study the mechano-chemical coupling of myosin-10, we used the *in vitro* actin filament sliding assay and a dual-beam, optical tweezers apparatus to perform single molecule mechanical studies to measure displacements in the nanometer and forces in the piconewton regime. To complement the single molecule myosin-10-HMM data, we performed bulk solution studies. In the actin sliding assay, the surface of a coverslip was coated with myosin-10 and fluorescently labeled actin filaments were allowed to move over the surface, powered via ATP. Myosin-10-HMM moves filaments at a velocity of ~410 ± 190 nm s⁻¹, similar to the speed of intact, single GFP-tagged, myosin-10 moving within filopodia of live mammalian cells at room temperature (~600 nm s⁻¹) measured via total internal reflection fluorescence microscopy and analyzed using single-particle tracking. The three-bead optical tweezers assay, whereby a single fluorescently labeled actin filament was attached between two optically trapped 1 μm polystyrene beads via a Neutravidin-biotin linkage, and lowered on to a third pedestal, sparsely coated with myosin-10-HMM, was used to measure mechanical and kinetic parameters. The results revealed that the average powerstroke size was ~17 nm with an single actomyosin-10 stiffness of ~0.34 ± 0.09 pN/nm, with an ADP release limited actomyosin-10-HMM detachment rate of ~ 13 s⁻¹, which was also in good agreement to both steady-state and transient kinetic data. In most of the raw data traces, we observed displacements of unitary size, however at low ATP concentrations (~500 nM) and low optical trap stiffness (<0.005 pN/nm) we also observed a number of interactions that exhibited multiple, staircase-like movements consisting of up to 5 or 6 steps per binding interaction. This behaviour is characteristic of a *processive* molecular motor. We will discuss these measurements in the context of structure, mechano-chemical coupling and the functional significance of this motor in the living cell.

Not All C57bl/6 Mice Are Created Equal. M. Bourdi, J. S. Davies, K. Sendide, and L. R. Pohl, Laboratory of Molecular Immunology

C57BL/6 inbred mouse strain is one of the most widely used animals for research models. However, their popularity has led to the creation of several C57BL/6 mice substrains maintained within and among different vendors. In this regard, major discrepancies between C57BL/6 mice substrains have been shown in several areas of research including behavioral studies, diabetes, cancer and oxidative stress, among others. We present evidence here describing similar problems in the field of toxicology. When a hepatotoxic dose of acetaminophen (APAP) was administered to substrains of C57BL/6 mice from 4 different vendors (Taconic Farms, Charles River, Harlan and The Jackson Laboratories), significant differences were found in their susceptibility to liver injury and survival. Comparing APAP bioactivation of C57BL/6J (The Jackson Laboratory; the least susceptible substrain) with C57BL/6N Tac (Taconic Farms; one of the most susceptible substrains), we found by immunoblot analysis a reduced level of mitochondrial APAP protein-adducts in C57BL/6J mice compared to C57BL/6NTac that was correlated with mitochondrial levels of CYP2E1. Moreover, APAP treatment caused less mitochondrial glutathione (GSH) depletion in C57BL/6J mice compared to C57BL/6NTac. Interestingly, the levels of APAP protein-adducts and GSH in whole liver homogenates did not differ significantly between the two substrains. Overall, these findings suggest, for the first time, that susceptibility differences exist between different C57BL/6 mice substrains in APAP-induced liver injury (AILI) model and possibly other forms of injury. It also stresses that researchers should carefully consider the appropriate C57BL/6 mice substrain for control when using genetically engineered mice on a C57BL/6 background, not only for toxicological research, but also for other biomedical studies.

Computational Prediction of *Drosophila* Muscle Enhancers.

B. W. Busser¹, L. Taher², A. A. Philippakis³, M. L. Bulyk³, I. Ovcharenko² and A. M. Michelson¹, ¹Laboratory of Developmental Systems Biology, ²Computational Biology Branch, National Center for Biotechnology Information, and ³Division of Genetics, Department of Medicine, Brigham and Women's Hospital, Boston, MA.

Embryonic muscle founder cells (FCs) in *Drosophila* develop under the influence of multiple signal-activated and tissue- and cell-specific transcription factors (TFs). We have previously defined a transcriptional regulatory model for a subset of FC genes. To extend this regulatory model to all FC genes, we have applied a classifier that identifies *cis* regulatory modules (CRMs) based on the presence or absence of various sequence features including known and putative transcription factor binding sites. To apply the classifier, we first increased the number of FC CRMs by incorporating orthologs having 50-75% identity from other *Drosophila* species. *In vivo* reporter assays established that many of these orthologous CRMs are functional in *D. melanogaster*. We next applied the classifier and found that it performed with high sensitivity and specificity. In addition, the top-scoring predictions were heavily enriched for FC genes when the classifier was run genome-wide to search for new FC CRMs. As an initial test of the accuracy of these predictions, we found that genes associated with top-scoring candidate FC enhancers were expressed in muscle FCs. Finally, many of the binding motifs learned by the classifier are recognized by TFs known to regulate muscle FC genes, and novel motifs have suggested plausible new

candidate TFs in the myogenic regulatory network. In conclusion, these studies are providing novel insights into the transcriptional codes that regulate gene expression during muscle development.

Phenotypic Characterization of Disseminated Cells with *TSC2*-related Loss of Heterozygosity in Patients with Lymphangiomyomatosis. X. Cai, G. Pacheco-Rodriguez, Q. Y. Fan, M. Haughey, L. Samsel, S. El-Chemaly, H. P. Wu, J. P. McCoy, W. K. Steagall, J. P. Lin, T. N. Darling, J. Moss, Translational Medicine Branch

Lymphangiomyomatosis (LAM), occurring sporadically (S-LAM) or in patients with tuberous sclerosis complex (TSC-LAM), results from abnormal proliferation of smooth muscle-like LAM cells exhibiting mutations or loss of heterozygosity (LOH) of the TSC genes, *TSC1* or *TSC2*. Here we identified molecular markers useful for isolating LAM cells from body fluids and determined the frequency of *TSC1* or *TSC2* LOH. Candidate cell surface markers were identified using gene microarray analysis of human *TSC2*^{-/-} cells. Cells from bronchoalveolar lavage fluid (BALF), urine, chylous effusions, and blood were sorted based on reactivity with antibodies against these proteins (e.g., CD9, CD44v6) and analyzed for LOH using *TSC1*- and *TSC2*-related microsatellite markers and single nucleotide polymorphisms (SNPs) in the *TSC2* gene. CD44v6⁺CD9⁺ cells from BALF, urine, and chyle showed *TSC2* LOH in 80%, 69%, and 50% of patient samples, respectively. LAM cells with *TSC2* LOH were detected in over 90% of blood samples. LAM cells from different body fluids of the same patients, showed, in most cases, identical LOH patterns, that is, loss of alleles at the same microsatellite loci. In a few S-LAM patients, LAM cells from different body fluids differed in LOH patterns. No S-LAM patients with *TSC1* LOH were identified, suggesting that *TSC2* abnormalities are responsible for the vast majority of S-LAM cases, and that *TSC1*-disease may be subclinical. Our data support a common genetic origin of LAM cells in different sites in most S-LAM cases, consistent with a metastatic model; they also suggest, however, that LAM cells could arise from different clones.

Telomere Shortening Promotes Chromosomal Instability and Predicts Malignant Clonal Evolution in Aplastic Anemia.

R. T. Calado¹, J. N. Cooper¹, P. Scheinberg¹, C. Wu¹, M. A. Zago², H. Padilla-Nash³, T. Ried³, E. M. Sloand¹ and N. S. Young¹, ¹Hematology Branch, ²Department of Internal Medicine, University of Sao Paulo at Ribeirao Preto Medical School, Ribeirao Preto, Brazil, ³Genetics Branch

In murine models, telomere erosion promotes chromosomal instability via breakage-fusion-bridge cycles, contributing to the early stages of tumorigenesis. However, direct evidence that short telomeres predispose to cancer development in humans is lacking. In acquired aplastic anemia, evolution to malignant clonal disorders is a major complication after immunosuppressive therapy, affecting up to 15 percent of patients at 10 years. We investigated whether telomere length measured at diagnosis predicted clonal evolution in these patients. Telomere length was measured from DNA extracted from peripheral blood leukocytes collected at disease presentation in 183 consecutive patients enrolled in successive clinical trials for immunosuppressive regimen as first line therapy for severe aplastic anemia at the Clinical Research Center, National Institutes of Health (ClinicalTrials.gov identifier numbers, NCT00001964, NCT00260689, and NCT00061360) and 164 healthy volunteers. Leukocyte telomere length of aplastic anemia patients at diagnosis was in the normal

range and was not shorter than in healthy controls (ANOVA-F test). Telomere length was corrected for age and patients were separated into two groups: patients with short telomeres (in the lowest quartile) and long telomeres (other quartiles). Telomere length was a critical and independent predictive biomarker for evolution to myelodysplastic syndrome, especially monosomy 7, and acute myeloid leukemia (AML) in patients with acquired aplastic anemia (Multivariate Cox Proportional Hazard Model, $P=0.006$). Patients with short telomeres had six-fold higher probability to develop clonal malignant disease than did patients with longer telomeres. Bone marrow cells of aplastic patients were cultured in vitro for short term in the presence of cytokines and high-dose granulocyte-colony stimulating factor (G-CSF) and cells of patients with short telomeres ($n=5$) showed increased telomere-free chromosomal ends in comparison to cells of patients with long telomeres ($n=6$), by fluorescence in situ hybridization (FISH; $P<0.0001$). Spectral karyotyping (SKY) revealed that cultured bone marrow cells of patients with short telomeres exhibited aneuploidy and translocations, including Robertsonian translocations, which were not found in cells of patients with long telomeres. Bone marrow cells at diagnosis were further evaluated for the presence of monosomy 7 cells using interphase FISH in 73 patients. Telomere length inversely correlated with the frequency of monosomy 7 cells: the shortest the telomeres, the highest the percentage of aneuploid cells at diagnosis (Pearson $r=-0.5110$; $P=0.0009$). We further employed bone marrow cells of clinically healthy individuals carrying loss-of-function telomerase mutations and with extremely short telomeres ($n=5$) as a model for telomere dysfunction in hematopoietic cells in the absence of human disease. In vitro culture of these cells yielded aberrant karyotypes by SKY, including translocations and aneuploidy, and end-to-end chromosomal fusions by FISH. These results indicate that telomere length at diagnosis predicts evolution to myelodysplasia and leukemia in patients with acquired aplastic anemia treated with immunosuppression. Our findings support the hypothesis that short and dysfunctional telomeres restrain stem cell proliferation and predispose for malignant transformation by selecting stem cells that are prone to chromosomal instability. This is the first prospective study to demonstrate that short telomeres in human hematopoietic cells promote chromosomal instability in vitro and predispose to malignant transformation in humans.

Dna Damage Responses in Brca1 Deficiency Induced Apoptosis, Senescence and Tumorigenesis. L. Cao^{1,2}, C. X. Deng² and T. Finkel¹, ¹Translational Medicine Research Branch, NHLBI, ²Genetics of Development and Diseases Branch, NIDDK

Maintenance of genomic integrity by the DNA damage response (DDR) network is considered to be an important mechanism for protection of neoplastic transformation and premature aging. Recent studies showed that DNA damage and activation of DDR associate with senescence, aging, and pre-neoplastic transformation. However, the role of genomic instability/DNA damage and DDR in the causal links between aging and tumorigenesis remain elusive.

BRCA1 (Breast Cancer Gene 1) is an important checkpoint and DNA damage repair gene that is required for maintaining genomic integrity. The BRCA1 deficiency triggers senescence, apoptosis and embryonic lethality through activation of DDR (ATM-Chk2-p53/53BP1-p53) signaling, which acts as a natural selection to eliminate mutilations. By introducing mutation of DNA damage response genes into a mouse model missing the BRCA1, we evaluated the DDR in vivo under genotoxic stress

caused by BRCA1 deficiency. Conversely, the absence of these genes allows BRCA1 mutant mice survive to adulthood at a variable risk of tumorigenesis and premature aging. Thus, in response to DNA damage caused by BRCA1 deficiency, the activation of the DDR (ATM-Chk2-p53/53BP1-p53) signaling pathway contributes to the suppression of neoplastic transformation, while leading to compromise organism homeostasis, and highlight how accurate maintenance of genomic integrity is critical for the suppression of both aging and malignancy, and provide a further link between aging and cancer.

Characterization of Integrin “Puffs”: a Dynamic Subset of Ecm-bound Integrins in Adherent Tissue Cells that are Not Associated with Focal Adhesions. L. B. Case and C. M. Waterman, Laboratory of Cell and Tissue Morphogenesis

Integrins are transmembrane $\alpha\beta$ heterodimers that bind ligands in the extracellular matrix (ECM) to mediate cell adhesion and signaling. Activated integrins in adherent mesenchymal cells bind ligand and cluster together into large multiprotein complexes called focal adhesions (FA). Clustering of integrins is thought to be critical to their ability to bind ligand and to their roles in adhesion and signaling. However, we found using Total Internal Reflection Fluorescence (TIRF) single molecule imaging of adherent cells that about half of ECM-bound integrins are not found in visible FA clusters. To further examine the dynamics of unclustered integrins, we used TIRF to image live human osteosarcoma (U2Os) cells expressing fluorescently tagged α_v integrin. We found that outside of FAs, integrins appear in transient crescent or amorphous shaped “puffs” of much lower density and generally larger area ($\sim 16-40 \mu\text{m}^2$) than integrins in FA clusters. Integrin puffs are highly dynamic, propagating around the ventral plasma membrane at velocities between 0.74 and 5.7 $\mu\text{m}/\text{min}$ with lifetimes of 1-5 min, and they are never observed to develop into FA. We coexpressed tagRFP labeled integrins with other FA proteins fused to GFP. Actin precedes integrin in puffs by 3 minutes. After actin, zyxin and VASP appear, followed by paxillin, vinculin, talin and, finally, integrin. Furthermore, puffs are dependent on actin polymerization, as treatment with Latrunculin A eliminates puffs. Our data suggests that actin polymerization at the ventral cell surface leads to inside-out activation and transient binding of unclustered integrins to the ECM. We conclude that this represents a novel and perhaps functionally distinct class of integrins from those in FAs.

A Non-proteasomal Catabolic Pathway for Oxidized Protein – IRP2 as a Model System. A. H. K. Chang, J. Jeong, R. L. Levine, Biochemistry and Biophysics Center

Protein oxidation and turnover have been implicated as important factors in aging and age-related diseases. Iron regulatory protein 2 (IRP2) is an excellent model system to study this process, because it is known to bind iron/heme and undergoes oxidation and degradation. IRP2 is also pivotal in the post-transcriptional regulation of genes involved in iron homeostasis. The ubiquitin-proteasome system is thought to be responsible for IRP2 turnover. However, by employing a specific monoclonal antibody, fluorophore-labeled secondary antibody, and infra-red imaging system, we report here that in human 293A cells the degradation of endogenous IRP2 is mediated by a novel non-proteasomal mechanism. Epoxomicin, a relatively specific proteasome inhibitor, caused a decrease of IRP2 steady-state protein level after a 6h treatment – opposite to what would be expected if proteasome mediated the degradation. Quantitative RT-PCR

showed that the decrease was not due to diminished mRNA level. Time-dependent analysis of IRP2 after cycloheximide-mediated inhibition of protein synthesis established that IRP2 protein degradation was accelerated by epoxomicin. Neither gene chip analysis nor a panel of protease inhibitors identified a candidate as the non-proteasomal IRP2 protease. However, the latter led us to discover that the activation of the non-proteasomal system required calcium. Thapsigargin decreased IRP2 level, and epoxomicin-induced IRP2 degradation was inhibited by calcium channel blockers PD150606, TMB-8, and intra-cellular calcium chelator BAPTA-AM. We conclude that under our experimental conditions in 293A cells, a non-proteasomal protease system functions in the degradation of IRP2. Identification of this mechanism would have a profound impact on the field of protein turnover and aging.

Modeling the Genetic Basis for Human Congenital Heart Disease Using Forward Genetic Screening with Mouse ENU Mutagenesis. B. Chatterjee, Q. Yu, Y. Shen, S. Sabol, L. Leatherbury, C.W. Lo, Laboratory of Developmental Biology

Congenital heart disease (CHD) is one of the most prevalent birth defects, affecting up to 5% of live births. To examine the genetic contribution to CHD, we used mice from a genome wide mutagenesis screen to recover mutations causing CHD. The availability of genetically homogenous inbred mouse strains and well-tested mutagenesis protocols make mice a compelling model system for pursuing cardiovascular genetic studies. In addition, mice, like humans, have 4 chamber hearts with separate pulmonary and systemic circulation - structures that are the major targets of CHD. Using noninvasive fetal echocardiography, we developed a high throughput cardiovascular phenotyping protocol and scanned over 13,000 C57BL6 (B6) mouse fetuses. We identified all major cardiovascular defects in this screen. Together they exhibited most of the major CHD found clinically. This included all varieties of outflow and atrial/ventricular septation defects, single ventricle and other chamber defects, as well as aortic arch and various venous anomalies. We mapped fifteen mutations, and identified 11 of the mutant genes. The mutations were recovered using several different strategies including microarray gene expression profiling, bulk exon sequencing using cDNA and genomic DNA, and massively parallel sequencing of BAC contigs or sequence captured DNA spanning the mutation containing map interval. Genetic modifier effects were commonly observed, a problem that was effectively managed using consomic mouse lines for recovery of the B6 genetic background. Although DNA resequencing cost has dramatically decreased, recovery of the genome interval for DNA resequencing remains costly and rate limiting, a problem that may be addressed by emerging technologies. Overall, we showed 7 of the mutations were in genes not previously known to cause CHD, with 2 being entirely novel genes with no known function. Surprisingly, 6 of the mutations were in genes required for ciliogenesis. Given our experience, we propose ENU mutagenesis and fetal echocardiography can be used to achieve a saturation screen of the mouse genome to recover the majority of genes causing CHD. Such studies may provide novel insights into the developmental etiology of CHD, and provide the basis for constructing a genotype-phenotype-outcome matrix to guide clinical care of patients with congenital heart disease.

Clinical Development of Panitumumab-CHX-A''-DTPA as a Potential SPECT Diagnostic Imaging Agent. S. Cheal, O. Vasalatiy, S. Cofiell, A. Bate, J. L. Tatum, G. L. Griffiths, The

Imaging Probe Development Center at NHLBI in collaboration with NCI

Panitumumab (Vectibix[®], Amgen, Inc., Thousand Oaks, CA), the first fully humanized monoclonal antibody targeting the epidermal growth factor receptor (EGFR), is currently approved for metastatic colorectal cancer with disease progression on or following standard chemotherapy. EGFR overexpression is detected in many human cancers including those of the colon and rectum, but EGFR is also constitutively expressed in many epithelial tissues (e.g. skin follicle). Thus, skin problems including skin swelling, blisters, and abscesses are common following pPanitumumab administration, and fatal systemic infections have been reported. Also, there is a very poor/low response rate to therapy (10% alone, and 20% when used in combination with chemotherapy) making the risk-benefit estimation vitally important with regard to decisions regarding Panitumumab therapy. There is currently no reliable predictor of EGFR antibody efficacy. To this end, the development of a pPanitumumab SPECT imaging agent that will demonstrate demonstrated retained immunoreactivity and tumor localization *in vivo* will be developed as a predictor of likely efficacy. This will be accomplished by chemically conjugating the bifunctional chelate *N*-[2-amino-3-(*p*-isothiocyanatophenyl)propyl]-*trans*-cyclohexane-1,2-diamine-*N*, *N'*, *N''*, *N'''*, *N''''*-pentaacetic acid (CHX-A''-DT-PA), to the antibody at well defined and limited substitution ratios. Subsequently we prepared an In-111 radiolabeling with In-111 ([γ : 0.171 MeV (90%), 0.245 MeV (94%)]) radiolabeled Panitumumab in > 98% radiochemical yield. The preparation of pPanitumumab-CHX-A''-DTPA Ab-conjugate, its radiolabeling, and *in vitro* analysis and testing immunoreactivity will be discussed. Results establish the feasibility to reproducibly prepare Panitumumab-CHX-A''-DTPA, radiolabel with high efficiency and purity, and maintain *in vitro* activity following addition of In-111. The agent is under development for preclinical evaluation, and later clinical application as a specific imaging agent for the presence of EGFR positive cancers.

Mice with reduced mTOR Expression have Altered Cellular Metabolism and Age Slower. E. Chen, J.J. Wu, J. Lui, L. Cao, M. Allen, D. Springer, B. Mock, and T. Finkel, Translational Medicine Branch

The mammalian Target of Rapamycin, mTOR, is a serine/threonine kinase that is a central regulator for cell growth and metabolism. It has been shown that mTOR-mediated metabolic processes contributes to aging. Whole body ablation of mTOR in mice is embryonic lethal, and thus the physiological role of mTOR has not been examined directly. In our studies, we are using a novel mouse model, mTOR^{-/-}, that has reduced mTOR expression due to insertion of a neomycin cassette into the intron region of the gene. Using this model, we are testing the hypothesis that low levels of mTOR expression affects metabolism and extends lifespan by slowing the aging process. To investigate mTOR-mediated regulation of cellular metabolism, mouse embryonic fibroblast (MEFs) cells were generated from wild-type (WT) and mTOR^{-/-} embryos. We found that mTOR^{-/-} MEFs have reduced growth rates, but similar rates of cell death when compared to WT MEFs. Additionally, mTOR^{-/-} MEFs have reduced whole cell respiration, with a coinciding increase in their glycolytic rates. However, FACS and QT-PCR analyses showed that the mitochondrial number were similar between the WT and mTOR^{-/-} MEFs, suggesting that mTOR is required to regulate the

activity of the mitochondria. Consistent with reduced mitochondria-mediated respiration, mTOR^{-/-} MEFs have reduced intracellular reactive oxygen species (ROS) levels. Since ROS generation has been associated with an increased rate of senescence, we examined the rate of senescence in primary MEFs. mTOR^{-/-} MEFs have lower rates of senescence than their WT counterparts. To examine the *in vivo* role of mTOR in aging-mediated processes, we subjected aged WT and mTOR^{-/-} mice to a rotarod test. Our preliminary findings show that aged mTOR^{-/-} mice perform better physically than their aged WT controls. Taken together, our results indicate that systematic reduced levels of mTOR expression altered cellular metabolism and respiration, which can potentially contribute to slowing the aging process.

Patient-specific Disease Modeling In Vitro, Using Induced Pluripotent Stem Cell Technology. G. Chen, A. Walts, M. Ma, S. Holland, M. Boehm, Translational Medicine Branch

The ability to study human disease mechanisms *in vitro* is complicated by the fact that tissue or cells affected in a particular disease are often unavailable. Induced pluripotent stem cell (iPS) technology has drastically changed the field of human disease modeling. The iPS cells self-renew and retain the potential to be differentiated into all cell types, providing promise in the field of regenerative medicine. Here, we show that multiple human iPS cells were obtained from HUVEC cells from both health donor and patients with hyper-IgE syndrome by lentivirus-mediated infection of four transcription factors (Oct4, Sox2, Klf4, and c-Myc) in feeder-free culture condition. The iPS cells displayed flat and tightly packed morphology similar to human embryonic stem cells (ESCs), with high nucleus/cytoplasm ratio, and prominent Nucleoli. These cells can be further expanded in both feeder and feeder-free conditions, with growth rates comparable to that of human ESCs. We analyzed the expression of undifferentiated ESC marker by immunocytochemistry and flow cytometry, including pluripotent markers alkaline phosphatase, Oct4, NANOG and ESC-specific surface markers SSEA-4 and TRA-1-60. These results indicate that the expression of pluripotent markers in these cells is shared with human ESCs. Moreover, these iPS cells were able to form embryoid body in suspension culture with serum. The colonies will provide an unlimited cell resource and offer a new platform for insights into pathogenesis of hyper-IgE syndrome *in vitro*, drug discovery, and autologous cell replacement therapy. The future investigations will address further characterization of these cells, such as differentiation potential *in vivo* and *in vitro*, and genetic and epigenetic status.

Syndecan-3 Regulates Zebrafish Pigmentation. T-Y. Choi, K. Kramer, Lab of Developmental Biology Group

Syndecan-3 (Sdc3) is a membrane-bound heparan sulfate proteoglycan that is thought to function as a co-receptor for growth factors and cytokines. Mice lacking Sdc3 have reduced food intake and body weight, and further work supports a hypothesis wherein Sdc3 regulates energy balance by modulating the ability of the agouti protein AGRP to competitively antagonize α -Melanocyte Stimulating Hormone (α -MSH) binding to the melanocortin-4 receptor in the hypothalamus. However, this hypothesis has not been directly tested. Another agouti protein, ASIP, is a potent antagonist of α -MSH developing melanocytes, neural crest-derived cells that produce melanin pigments in various tissues including skin, hair and eye. To investigate a possible role for Sdc3 in melanogenesis, zebrafish embryos were injected with morpholinos designed to knockdown endogenous *zsdc3*.

Melanocyte development appeared normal in *zsdc3* morphant zebrafish; yet pigmentation was decreased and could be ablated at high morpholino doses, a phenotype that was rescued by co-injection of *zsdc3* mRNA. The results suggest that Sdc3 regulates melanin synthesis and allows for the direct testing of the co-receptor hypothesis in a tractable model system.

Targeted Disruption of PDE3B Moderates Atherosclerosis in Apolipoprotein E^{-/-} and LDL-R^{-/-} Mice. Y- W. Chung¹, S. C. Hockman¹, Y. Tang¹, M. J. Amar², A. T. Remaley², and V. C. Manganiello¹,¹Translational Medicine Branch and ²Vascular Medicine Branch

Apolipoprotein E knockout (apoE^{-/-}) mice develop spontaneous hypercholesterolemia and atherosclerosis. Inflammatory infiltrates in the atherosclerotic plaques contain cholesterol-laden macrophages ("foam cells") and T lymphocytes. These and other inflammation-related cells are presumably responsible for the increased circulating levels of proinflammatory cytokines, interferon- (INF-) and tumor necrosis factor- (TNF-), as well as macrophage-derived interleukin (IL)-12 and IL-18, in apoE^{-/-} mice. Interestingly, we found that targeted disruption of PDE3B was associated with decreased macrophage "markers" in epididymal white adipose tissue (EWAT). Moreover, chemokine (C-C motif) ligand 2 (CCL2) and its receptor CCR2, which play an important role in macrophage chemotaxis, were less highly expressed in EWAT of PDE3B^{-/-} mice than WT mice. In addition, after lipopolysaccharide (LPS) injection, plasma levels of TNF- α , IL-12 and IL-18 were lower in PDE3B^{-/-} mice than WT mice. To examine the possible effect of PDE3B on macrophage infiltration and atherosclerotic plaque formation, apoE^{-/-}/PDE3B^{-/-}, as well as LDL-R^{-/-}/PDE3B^{-/-} mice were generated. Compared to apoE^{-/-} and LDL-R^{-/-} mice, in the aorta of apoE^{-/-}/PDE3B^{-/-} (normal diet) and LDL-R^{-/-}/PDE3B^{-/-} (Western diet high in fat for 5 months) mice, plaque formations was significantly reduced, respectively, suggesting a role for PDE3B in modulating the inflammatory response and suggesting that PDE3B signaling pathways might provide possible therapeutic targets to moderate atherosclerosis.

Epidemiology of Vaso-occlusive Pain in Sickle Cell Anemia and Its Association with a Susceptibility Marker in the GCHI Gene. D. Darbari, I. Belfer, V. Youngblood, K. Desai, L. Diaw, L. Freeman, M. Hildeshem, C. Minniti, V. Nolan, J. N. Milton, S.W. Hartley, M. H. Steinberg, D. Goldman, M.B. Max, G. Kato, J. G. Taylor VI, Sickle Cell Disease Vascular Disease Unit, Pulmonary and Vascular Medicine Branch

The reasons for variability in pain severity in sickle cell anemia (SCA) have not been clearly elucidated. Recently a GTP cyclohydrolase (*GCHI*) genetic variant was shown to affect sensitivity to pain in a non-SCA population via tetrahydrobiopterin (BH4), possibly through Nitric oxide (NO) dependent pathways. BH4 is an essential cofactor for NO synthesis and lower BH4 is associated with pain protective phenotype in non-SCA population. The present study was designed to define the epidemiology of pain in SCA and its association with variants of *GCHI* using NIH and CSSCD cohorts. Subjects were classified into low pain (zero hospitalizations per year; n= 104) or high pain (≥ 1 hospitalizations/year; n=160) groups. The low pain group had lower hemoglobin (P=0.0007) and HDL (P=0.02), but higher LDH (P=0.004), ferritin (P=0.009) and a higher proportion of individuals with tricuspid regurgitation jet velocities (TRV) ≥ 2.5 m/s (56.6 % vs 42.4% P= 0.03). Mortality during follow-up occurred at an earlier age in those with high pain (P=0.002). This

pain phenotype was associated with a *GCHI* haplotype defined by polymorphism rs8007267 (OR 2.51, $P=0.008$), and this finding was also replicated in the CSSCD cohort (OR 2.2; $P=0.0088$). Cell lines from the Yoruban Hapmap homozygous for the *GCHI* rs8007267 haplotype had higher *GCHI* mRNA ($P=0.02$) and protein expression. We conclude that *GCHI* genetic variants contributes to pain sensitivity in SCA possibly through BH4 and NO dependent pathways and might be a target for future therapies designed to reduce pain in SCA.

Measurement of Human Plasma Nitrate and the Possible Correlation to Endothelial Dysfunction and Hemolysis. R. Dave, X. Wang, L. Mendelsohn, G. J. Kato, Pulmonary and Vascular Medicine Branch

Nitric oxide (NO) is a reactive gaseous molecule that plays a critical role in both physiological and pathological vascular signaling. It is thought that the majority of the NO that does not diffuse abuminally reacts rapidly ($k = 2 \times 10^7 \text{ M}^{-1} \text{ s}^{-1}$) with hemoglobin by the following equations: $\text{NO} + \text{oxyhemoglobin (Fe}^{2+}) \rightarrow \text{nitrate (NO}_3^-) + \text{methemoglobin (Fe}^{3+})$
 $\text{NO} + \text{deoxyhemoglobin (Fe}^{2+}) \rightarrow \text{iron-nitrosyl hemoglobin (Hb-NO)}$

Nitrate is then a stable molecule in plasma and can be measured, once drawn from the patient, without the use of any preservation solutions. I measured the nitrate levels in plasma from Sick Cell patients. These levels were measured using vanadium(III) in 1N hydrochloric acid with the reaction chamber heated to a temperature of 90 c. The vanadium reduces the nitrate to NO gas, which is released in the closed system apparatus of the ozone-based chemiluminescent assay. The samples were precipitated with ethanol and then loaded into the vanadium. The resulting peaks were analyzed using Origin 6.1 software. I am in the process of correlating that data to our lab's previous endothelial dysfunction, haptoglobin and hemoglobin data, and will present the results.

Alterations in Cardiac microRNA Expression Between Male and Female Mice. A. M. Deschamps¹, R. Wang², D. Liu², N. Raghavachari², E. Murphy¹, ¹ Translational Medicine Branch and ²Microarray Core

Susceptibility and/or degree of pathology in a number of cardiovascular diseases differ between males and females. A widely-held belief is that the female hormone, estrogen, is responsible for this observed disparity. However, the mechanism by which estrogen induces these differences is not well-defined. It is thought that alterations in gene expression might be the source for male-female differences. One mechanism of gene regulation is through the expression of microRNAs. MicroRNAs are small (20-24 nucleotides) single stranded pieces of non-coding RNA that bind to mRNA and disrupt translation. Accordingly, the hypothesis of this study was that differential microRNA expression would account for gender differences in cardiovascular disease susceptibility. Using an Affymetrix microRNA array and mRNA array, we observed some differences between male and female microRNA expression from principle component analysis. Specifically, we saw a reduction in miR-24 in females compared to males. We also discovered several miR-24 target mRNAs that were altered in male and female hearts such as transforming growth factor beta-induced and death effector domain containing protein. Thus, it is possible that inherent differences in miR-24 in female hearts compared to males may alter mRNAs and have a role in cardiovascular disease susceptibility.

Synthesis of UDP-2-Ketogalactose and UDP-2-Ketoglucose for the Site-Specific Linkage of Biomolecules via Modified Glycan Residues using Glycosyltransferases. A. E. Dulcey, J. Lamb, P. K. Qasba, G.L. Griffiths, The Imaging Probe Development Center at NHLBI in collaboration with NCI

Structural information on glycosyltransferases previously revealed that the sugar-donor specificity of these enzymes can be broadened to include modified sugars with chemical handles that can be utilized for conjugation chemistry. The potential of wild-type and mutant glycosyltransferases to produce glycoproteins with modified sugar moieties has made it possible to insert orthogonally reactive groups at specific protein sites. This platform technology enables the preparation of a range of site-specifically modified agents for targeted drug delivery such as drug-bearing bio/nanoparticles, and for diagnostic imaging modalities such as for magnetic resonance imaging. The first series of orthogonal groups we targeted was insertion of a ketone group on glycoproteins as this functional group is not abundant/present in biological systems. The ketone group insertion would then allow further substitution via a mild Schiff base or aminoxy-modification strategy. The synthesis of UDP-2-ketogalactose was previously carried out, albeit with great difficulty and low efficiency and the principal investigator at NCI approached the IPDC for assistance with this agent and for synthesis of the heretofore non-described 2-ketoglucose analog. The synthetically modified approach developed by the IPDC for the synthesis of UDP-2-ketoglucose and UDP-2-ketogalactose has allowed improved access to the desired compounds. The UDP-2-ketoglucose analog was synthesized in 8 steps and 4.7% overall yield, and the UDP-2-ketogalactose analog was obtained in the same number of steps with an overall yield of 5.3%. The yield of the latter represents an approximately eight-fold improvement over the 1st generation literature synthesis, which involved 8 steps and an overall yield of 0.7%.

Adeno-associated Virus-2 (AAV2) Integrase Mediates Specific Integration to Locus Aavs1 on Chromosome 19q13.3. A. E. Dunfee, J. R. Clevenger, C. E. Dunbar, and A. Larochelle, Hematology Branch

Gene therapy has already demonstrated considerable efficacy in the treatment of hematopoietic disorders such as X-SCID, ADA-SCID and CGD. However, the retroviral vectors utilized in these therapies can integrate into the host genome in a semi-random fashion, which can result in dysregulated expression of nearby genes, including many which may lead to cancer. In an effort to avoid insertional mutagenesis, we investigated the use of a selected integrase, the AAV2 integrase, to effect targeted integration in the genome of human hematopoietic cells. This integrase is known to result in site-specific integration in the genomic locus AAVS1 located on chromosome 19q13.3, a region of the genome not associated with cancer formation, providing a potentially safer approach for gene therapy of hematopoietic disorders. To test this approach, we transfected the human hematopoietic cell line K562 by nucleofection with two plasmids: one ts plasmid containing the GFP reporter gene flanked by the AAV2 ITRs; and one plasmid containing the AAV2 integrase gene (AAV2 integrase group). Both the AAV2 integrase and GFP genes were under the control of the EF1 α promoter. Two control lines were also established by transfecting the same GFP plasmid in combination with a defunct AAV2 integrase plasmid (negative control group) or with a plasmid

expressing an integrase derived from the bacteriophage PhiC31 with no known specificity to chromosome 19q13.3 (PhiC31 integrase group). One day after transfection, 40% of K562 cells expressed GFP in both the AAV2 integrase and negative control groups, and 75% GFP+ cells were detected in the PhiC31 integrase group. Two months later, 2% and 1% of transfected cells maintained detectable GFP expression in the AAV2 integrase group and PhiC31 integrase group, respectively, while only 0.1% of the negative control group expressed GFP. GFP+ cells were subsequently sorted via FACS, cultured for up to 6 months and assessed by flow cytometry for GFP positivity at regular intervals. In the AAV2 integrase group, more than 98% of the sorted cells remained GFP+ after 6 months in culture compared to 40% in the negative control group. Sorted cells were assayed by PCR for detection of integration in locus AAVS1 using a forward primer specific to the integrated GFP gene and a reverse primer specific to various regions of the AAVS1 locus. A PCR product was detected only in the AAV2 integrase group, confirming specificity of integration at this locus. Additionally, sorted cells were assayed by FISH using dual hybridization with probes targeting GFP and chromosome 19 to detect integration to chromosome 19q. In 8 of 10 (80%) metaphases analyzed, integration was detected on chromosome 19q in cells from the AAV2 integrase group. In contrast, integration was observed in various loci in the PhiC31 integrase group but no integration (0/10) was detected on chromosome 19q. There was also no chromosome 19q integration (0/10) in the negative control group. This successful demonstration of site specific integration through the use of AAV2-integrase based vectors is a promising step towards safer clinical application of gene therapy for hematopoietic disorders.

MARCH Proteins Promote Delivery of Clathrin-Independent Endocytic Cargo Proteins MHCI and CD98 to Late Endosomes. C. Eyster, K. Viswanathan, K. Früh, and J. Donaldson, Laboratory of Cell Biology

Our lab has focused on studying the internalization and trafficking of plasma membrane proteins that enter cells through Clathrin-Independent Endocytosis (CIE), including major histocompatibility complex Class I protein (MHCI). In recently published work, we have used a proteomic-based screen of early CIE endosomes to identify new cargo proteins trafficking in the CIE pathway. Some identified cargo proteins followed a similar itinerary to MHCI, merging with components of the Clathrin-Dependent Endocytic (CDE) pathway in EEA1 positive early endosomes. CIE cargo is subsequently trafficked to late endosomes for degradation or recycled to the cell surface in CIE recycling tubes. Interestingly, a subset of new cargo proteins, typified by the protein CD98, did not traffic to EEA1 endosomes but were seen prominently in CIE recycling tubes. CD98 is a multifunctional protein, comprising the heavy chain of neutral amino acid transporters and interacting with integrins to promote cell adhesion. We have now shown that CIE cargos, like MHCI, that reach the EEA1 early endosome, accumulate after twenty-four hours in lysosomal structures if degradation is inhibited. However, cargos that do not reach the EEA1 compartment, like CD98, are largely absent from degradation depleted lysosomal compartments after twenty-four hours. Previous work from multiple labs has demonstrated that the viral proteins K3 and K5 can down regulate cell surface levels of MHCI in virally infected cells. K3 and K5 are membrane associated E3 ubiquitin ligases. Ten membrane-associated RING-CH (MARCH) proteins have been identified as human homologues of K3 and K5. It has been

previously shown that over-expression of MARCHIV in HeLa cells causes removal of MHCI from the cell surface likely through ubiquitination of the MHCI cytoplasmic tail. We have now shown that over-expression of MARCHIV enhances delivery of MHCI to lysosomes. Interestingly, over-expression of MARCHI, but no MARCHIV, causes a profound change in CD98 trafficking, causing it to now accumulate in late endosomes and lysosomes over short time periods. Therefore, MARCH proteins may play an important role in specifying CIE cargos for degradation, possibly through ubiquitination of their cytoplasmic tails.

Fatty Kidney is Associated with Hypertension and Chronic Kidney Disease: the Framingham Heart Study. M. C. Foster, S-J Hwang, J. M. Massaro, U. Hoffmann, C. S. Fox, Center for Population Sciences, Framingham Heart Study

Ectopic fat depots may mediate local and systemic disease. Significant increases in fat accumulation in the renal sinus are demonstrated in obese rabbits, which may be associated with hypertension and chronic kidney disease (CKD). The association of renal sinus fat and hypertension and CKD has not been previously studied in humans. The current analysis consists of 2923 participants who underwent computed tomography. Renal sinus fat and visceral abdominal fat (VAT) were quantified using computed tomography. Hypertension was defined using standard criteria. In a sub-sample (n=1210), CKD was defined as an estimated glomerular filtration rate <60mL/min/1.73m², as estimated using the cystatin C-based CKD-EPI equation. Fatty kidney was based on sex-specific 90th percentiles in a healthy referent sample. Hypertension and CKD were modeled as functions of fatty kidney using logistic regression. Hypertension models were adjusted for age, sex, current smoking, and then separately for body mass index (BMI) and VAT. CKD models were adjusted for age, sex, CKD risk factors, and then separately for BMI and VAT. The prevalence of fatty kidney was 30.1%. Fatty kidney was associated with an increased odds ratio (OR) for hypertension, which persisted after adjustment for BMI or VAT. Fatty kidney was associated with an increased OR for CKD after adjusting for CKD risk factors, which persisted after adjustment for BMI or VAT. These results demonstrate that fatty kidney is a prevalent condition associated with an increased risk of hypertension and CKD and suggest that renal sinus fat may play a role in blood pressure regulation and CKD.

Exosomes Derived from Antigen-pulsed Immature Dendritic Cells Attenuate Airway Inflammation and Hyperreactivity in a Murine Model of Allergic Asthma. K. Fredriksson, X-L. Yao, J. K. Lam, H. B. Bhakta, S. J. Levine, Pulmonary and Vascular Medicine Branch

Mature dendritic cells play a critical role in inducing effector T cells in allergic asthma, whereas immature DCs (iDCs) can mediate immunological tolerance. Exosomes are nanovesicles that originate from multivesicular bodies and are released from a variety of cell types, including DCs. Exosomes can transfer membrane proteins and cytoplasmic contents between cells, thereby serving as intercellular messengers that modulate immune responses. The aim of this study was to evaluate the effect of iDC-derived antigen-pulsed exosomes (iDC-Ex) on airway inflammation and hyperreactivity in a murine model of allergic asthma. Bone marrow was extracted from A/J mice and cultured with IL-4 and GM-CSF for 8 days. Rapamycin was added after 2 days and iDCs were pulsed daily with house dust mite (HDM) on days 4 - 8. Exosomes released into culture medium were collected

daily on days 5 - 8. Starting one week prior to the induction of asthma, mice received exosomes or saline by tail vein injection for a total of 4 weeks. Asthma was induced by daily intranasal administration of HDM 5 days/week during weeks 2 - 4. Mice treated with iDC-Ex showed a reduction in the total number of bronchoalveolar lavage fluid cells ($283 \times 10^4 \pm 38 \times 10^4$ vs. $61 \times 10^4 \pm 18 \times 10^4$, $p < 0.001$), as well as in the number of eosinophils ($211 \times 10^4 \pm 35 \times 10^4$ vs. $46 \times 10^4 \pm 13 \times 10^4$, $p < 0.01$). Plasma IgE levels in the treatment group were also significantly reduced ($6065 \text{ ng/ml} \pm 448$ vs. 3405 ± 369 , $p < 0.01$). iDC-Ex also significantly inhibited methacholine-induced airway hyperreactivity ($p < 0.01$). mRNA levels of Muc5ac and Gob5, which are associated with mucus hyperproduction and goblet cell hyperplasia, were significantly reduced in lungs from mice treated with iDC-Ex. mRNA levels of IL-17A, a pro-inflammatory cytokine produced by activated Th17 cells in asthma and IL-13, an important mediator of allergic inflammation secreted mainly by Th2 cells, were both significantly decreased in the iDC-Ex treated mice. Exosomes derived from antigen-pulsed iDCs attenuate airway inflammation and hyperreactivity in a murine model of allergic asthma. This suggests that exosome-based therapies might be efficacious for patients with allergic asthma.

Pon1 and Hdl in Sickle Cell Disease. L. A. Freeman, A. Tumblin, X. Wang and G. J. Kato, Pulmonary and Vascular Medicine Branch

Sickle cell disease (SCD) is caused by a mutation in the β -chain of hemoglobin, leading to sickle-shaped red blood cells (RBCs) that cause vasoocclusion. Major clinical features associated with vasoocclusion include acute pain crisis, acute chest syndrome and osteonecrosis. Additionally, RBC hemolysis is increased in a subset of SCD patients, leading to increased free hemoglobin and arginase in plasma and thus decreased NO bioavailability. Hemolysis in SCD has been linked to endothelial dysfunction and vasculopathy and is associated with pulmonary hypertension, leg ulceration, priapism and stroke (Kato et al. *Am J Hematol* (2009) **84**:618-625). The pathways affecting endothelial function in SCD have not yet been fully elucidated. As HDL and the HDL-associated lactonase PON1 are associated with vascular protection, we investigated HDL and PON1 in sickle-cell disease. Here we report that levels of both apoA-I and PON1 decrease in plasma of adult sickle-cell patients during pain crisis compared to their steady-state levels, as determined by Western analysis. PON1 enzyme activity also decreases significantly in crisis ($n=17$, $p < 0.02$). PON1 enzyme activity levels correlate significantly with apoA-I plasma levels ($p < 0.005$), consistent with previous reports that apoA-I and PON1 levels are coordinately regulated at the transcriptional level. HDL-C levels also decrease significantly during crisis ($n=18$, $p < 0.006$). Triglyceride levels did not change significantly during crisis; thus, pain crisis and PH are not analogous. In conjunction with previous reports on the protective role of PON1 and apoA-I towards the vasculature, our work suggests apoA-I and PON1 as potential therapeutic targets during pain crisis in SCD patients.

Visualization Of Dynamic Active Devices Via Adaptive Undersampled Projection Imaging in MRI-guided Interventional Procedures. A. K. George, C. E. Saikus, O. Kocaturk, R. J. Lederman, A. Z. Faranesh, Translational Medicine Branch

MRI-guided interventional procedures involve the manipulation of catheter-like devices guided by MRI instead of conventional X-ray. MRI-guided interventions promise to permit a new array of procedures previously attainable only with open surgical exposure, without the radiation exposure of X-ray. Accurate visu-

alization of the device is crucial to the success of the procedure. We introduce a method to visualize, from vastly reduced data, an active device with a "spatially extensive" signal (eg. a loopless coil), that is in motion. Using a small amount of data will allow for the seamless incorporation of the dynamic tracking of the 3D trajectory of the device into the existing imaging sequence. We reconstruct the 3D trajectory of a device from two undersampled 2D projection images. Prior information, i.e., the trajectory of the device at the previous time-instant, is used to reduce data requirements by adapting (a) the orientation and (b) the undersampling rate of the projection images collected at the current time instant. To compute the trajectory at a time-instant, we evaluate local 2D slices, which are perpendicular to the device trajectory at the previous time-instant, in the volume image formed from the product of the two projection images. We update the previous device trajectory by the location of the centroids of these local 2D slices and further refine the estimate by extending or cropping the ends of the device curve based on the re-projection of the 3D point onto each 2D projection image. We present in-vitro and in-vivo results.

Expression of SR-B1 in the Endothelium Enhances Reverse Cholesterol Transport. M. Ghias, B. Vaisman, A. Remaley, Pulmonary and Vascular Medicine Branch

Scavenger Receptor-BI (SR-BI) is a key player in cholesterol trafficking and lipoprotein metabolism. In hepatocytes, SR-BI is responsible for selective uptake of cholesterol ester (CE) from HDL, and thus promotes the biliary excretion of cholesterol. Besides hepatocytes, it has been shown that steroidogenic cells also possess the ability to selectively uptake CE, which is essential for steroidogenesis. Our studies show that SR-BI is highly expressed in aortic endothelial cells (EC). In order to further investigate the possible role of SR-BI in the endothelium, transgenic mice with mouse SR-B1 gene under control of an endothelial specific Tie2 promoter were created. The transgene was also transferred on apoE-Ko and SR-B1-Ko backgrounds. C57Bl6 wild type mice and SR-B1-Ko mice were placed on a high fat/high cholesterol diet for 3-6 months. Expression of SR-B1 in EC did not significantly change the plasma lipid and lipoprotein profiles in the various mouse models. Aortas of mice on apoE-Ko and wildtype backgrounds revealed that transgenic mice had $22 \pm 7\%$ and $37 \pm 14\%$ ($p < 0.02$) less aortic lesions, respectively than the control mice. To better understand the atheroprotective effects of SR-B1 in EC, we also studied the role of immortalized polarized EC in the selective uptake CE. Our experiments showed that HDL were able to penetrate through EC in direction from apical to the basolateral membrane. Similar to liver and adrenal cells, EC were able to selectively uptake CE from HDL mostly from basolateral compartment. In summary, these results reveal a role for SR-BI in maintaining cholesterol homeostasis in vasculature.

Phospho-specific Antibodies to Ser126 and Ser874 of NKCC2 Demonstrate Vasopressin Regulated Phosphorylation in Renal TAL. R. Gunaratne, T. Pisitkun, D.W.W. Braucht, L. Xie, M.M. Rinschen, J.D. Hoffert, C.L. Chou, M.A. Knepper, Epithelial Systems Biology Laboratory

Renal countercurrent multiplication is controlled in part through the action of vasopressin to regulate NaCl absorption in the TAL. Our recent phosphoproteomic analysis in rat has identified two predominant phosphorylation sites in the apical cotransporter NKCC2, viz Ser126 and Ser874. To investigate

effects of vasopressin, we made phospho-specific antibodies to both sites. Both antibodies specifically recognized the phosphorylated forms of NKCC2. Confocal immunofluorescence in rat showed TAL specific labeling, strongest in the cortical TAL. Immunoblotting of rat medullary TAL suspensions or homogenates from intact rat kidneys showed that vasopressin increases phosphorylation at both Ser126 (>8-fold) and Ser874 (>2-fold). Increases at both sites were blocked by H89 (10 μ M). *In vitro* assays revealed that purified protein kinase A can phosphorylate synthetic NKCC2 peptides at either Ser126 or Ser874. However, in the same assay, AMP kinase produced little or no phosphorylation at either site. In addition, we re-examined two previously demonstrated phosphorylation sites, viz Thr96 and Thr101, with the R5 double-phospho-antibody. Immunoblotting with 'cross-preadsorbed' R5 antibodies showed that vasopressin increases phospho-Thr96 (2-fold) but does not increase phospho-Thr101. These studies demonstrate regulated phosphorylation of NKCC2 at three sites in response to vasopressin.

Improving the Stability, Capability and Resolution of the Dual-Beam Optical Trap *In-vitro* Force Assay. S. Hernandez¹, Y. Takagi², E. E. Homsher^{2,3} and J. R. Sellers², ¹ Stony Brook University, Stony Brook, NY, ²Laboratory of Molecular Physiology, ³David Geffen School of Medicine at UCLA, Los Angeles, CA

Myosin is an essential biological molecular motor that is ubiquitous in all eukaryotic cells and plays a central role in nearly all physiological processes. A vast family of myosin exists, with each type of myosin having a specialized function in the cell. Different myosins have distinct ways of mechanically moving along the "track proteins" (for myosins, actin filaments function as "tracks") with different kinetic properties. Current research at the Laboratory of Molecular Physiology, NHLBI, NIH is focusing on deciphering the mechanism of the energy transduction and kinetics of these molecular motors in hopes of better understanding the physiological processes governed by their specific activities in the cell. This project aims to enhance the current methods used at the Laboratory of Molecular Physiology, to study the biomechanics and biochemistry of different types of myosins. Two of the main single molecule techniques for measuring the activities of myosin in our laboratory are optical trapping (OT) and total internal reflection fluorescence (TIRF) microscopy imaging. Using micro/nano-fabrication techniques to develop a novel experimental substrate for the actomyosin *in vitro* force assay will allow for the coupling of OT and TIRF microscopy for kinetic and chemical measurements. The new pedestal geometry features rectangular pedestals 2 μ m in width, 1.5 μ m in depth, 50 μ m apart. In addition, smaller (0.5 μ m vs. 1 μ m) polystyrene beads for optical trapping were used to increase temporal resolution of the *in vitro* force assay. To help reduce the low frequency noise exerted by air currents in the experimental room the optics for the OT system was shielded. The present project is currently in progress. The mask necessary for photolithography was developed, the protective cage around the current OT optical setup was completed, and covalent attachment of proteins used to attach the beads to the actin filament was completed for further testing and use in our laboratory. Future goals for the project include mass-production of the pedestals on quartz wafers, and the use of beads with higher index of refraction to increase the stiffness of the trap.

Landscape of Histone Variant H2A.Z in Mouse Embryonic Stem Cells. G. Q. Hu, K. Cui, Q. Tang, C. Crane-Robinson, K. Zhao, Laboratory of Molecular Immunology

Histone variants and their post-translational modifications have been implicated in transcriptional regulation in numerous organisms. H2A.Z is a histone variant evolutionarily conserved from yeast to humans. However, how H2A.Z and its post-translational modifications contribute to the regulation of transcription remains largely un-resolved. Here, we determined and compared the genome-wide profiles of H2A.Z and its N-terminal acetylated form in mouse ES cells. Our data revealed that the enrichment of H2A.Z is positively correlated with gene expression; H2A.Z in the promoters of active genes are highly acetylated. Notably, the majority of paused RNA polymerase II (Pol II) in promoters are co-localized with acetylated H2A.Z. Moreover, H2A.Z is co-localized with H3K4me3 but not H3K27me3 in both bivalent and active promoters. Of equal remarkable, binding sites of various transcription factors are concentrated in regions enriched in acetylated H2A.Zs. Our data also indicated that H2A.Z is localized to enhancers and insulators, with a higher acetylation in enhancers than insulators. Together, our data establish a genomic landscape of histone variant H2A.Z and its acetylated form in mouse ES cells and provide new insights into the function of H2A.Z in gene regulation.

STAT3 and Mitochondrial Function in the Hyperimmunoglobulin E Syndrome. R. Huang, K. Kim, C. Avila, A. Freeman, A. Aponte, M. Boehm, S. Holland, J. Milner, M Sack, Translational Medicine Branch

Mutations in signal transducer and activator of transcription 3 (STAT3) lead to the hyperimmunoglobulin E syndrome (HIES). As STAT3 has recently been shown to modulate mitochondrial function, we aimed to evaluate mitochondrial function in HIES cell lines. We first studied the in-vivo respiratory capacity of primary cells isolated from the solid tissue of HIES patients. All solid tissue-derived cells demonstrate a significant attenuation of respiratory capability in comparison to controls. As immune dysregulation is prominent in HIES, we next studied the respiratory profile of EBV-immortalized B-cells from HIES patients and bone-marrow mast cells (BMMCs) harvested from HIES-transgenic mice. Interestingly, in response to TNF- α stimulation, EBV-immortalized HIES B-cells demonstrate markedly increased oxidative capacity relative to control. Similarly, murine HIES-transgenic BMMCs also demonstrated increased respiratory capacity in response to antigen-induced degranulation. To elucidate a mechanism underlying these perturbations, we used two-dimensional gel electrophoresis and mass spectrometry to analyze the proteomic alterations in a STAT3 knocked-down human hepatoma line. Initial proteomic analysis reveals a perturbation in the post-translational phosphorylation of mitochondrial heat-shock proteins following Stat3 knock-down. Taken together, these data indicate that there is a phenotypic dichotomy between solid tissue as compared to bone-marrow derived cells from HIES patients in regards to oxidative capacity. Whether this divergence reflects the chronic activation of the immune system in HIES is being explored. The effect of modulation of mitochondrial HSP's to this program requires further investigation.

Constant pH Molecular Dynamics Simulations with the Replica-Exchange Method in the pH space. S. G. Itoh, A. Damjanoviæ, B. R. Brooks, Laboratory of Computational Biology

Protonation states of titratable groups in a protein depend on pH values of a solution. It is important to know the protonation states of the titratable groups on a pH value because the protein structures and functions are coupled to these states. In order to study the protonation states of the titratable groups with computer simulations, we propose a new computational method. This new method realizes effective sampling of protonation states in comparison with conventional constant pH simulations by combining those with the replica-exchange method. Consequently, pK_a values of titratable groups in proteins can be predicted more accurately by this new method than the conventional constant pH method.

Multiple Platelet Aggregation Genes are Identified by Genome-wide Association Meta-analyses. A. D. Johnson, L. R. Yanek, M. H. Chen, A. T. Kraja, M. G. Larson, R. Lin, G. H. Tofler, D.M. Becker, Q. Yang, M. A. Province, C. J. O'Donnell, L. C. Becker, Center for Cardiovascular Genomics, The Framingham Heart Study

Platelet aggregation provides both beneficial and harmful effects on human health, but few genes are known to contribute to variability in the process. No genome-wide association study has yet been reported for platelet aggregation phenotypes. We tested association of 2.5 million SNPs with platelet response to 3 agonists (ADP, epinephrine and collagen) in individuals of European ancestry ($N \leq 2,753$ in the Framingham Heart Study, $N \leq 1,232$ in the Genetic Study of Atherosclerosis Risk), with replication in an African-American cohort ($N \leq 840$ in the Genetic Study of Atherosclerosis). We identified associations of seven loci with platelet aggregation, near or in the *GP6* ($P = 4.6 \times 10^{-13}$), *PEAR1* ($P = 3.4 \times 10^{-12}$), *ADRA2A* ($P = 3.3 \times 10^{-11}$), *PIK3CG* ($P = 3.1 \times 10^{-9}$), *JMJ1DC* ($P = 1.6 \times 10^{-8}$), *MRVII* ($P = 2.0 \times 10^{-8}$), and *SHH* ($P = 4.5 \times 10^{-8}$) genes. Replication evidence was found for all loci. In total these findings provide new functional insights into platelet aggregation pathways and may suggest novel targeting mechanisms.

Three Dimensional Superresolution Fluorescence Microscopy Reveals Protein Stratification in Focal Adhesions. P. Kanchana-wong, G. Shtengel, A. M. Pasapera, Ericka B. Ramko, M. W. Davidson, H. F. Hess, C. M. Waterman, Laboratory of Cell and Tissue Morphodynamics

Focal Adhesions (FA) are complex protein assemblies that mechanically link the actin cytoskeleton to the extracellular matrix (ECM). Despite the central role of FA in cell motility and the wealth of biochemical and cell biological data on FA proteins, it remains unknown how these proteins are organized within FA. Here we used a 3-dimensional superresolution fluorescence microscopy technique, interferometric PhotoActivated Localization Microscopy (iPALM), to map 3D protein organizations in FA with <20 nm spatial resolution. Several key FA proteins are fused with photoactivatable fluorescent proteins and expressed in U2OS cells plated on a fibronectin-coated substrate. We find that within FA, integrins and actin are separated by a ~ 40 nm FA core, which consists of at least three protein-specific layers: integrin signaling layer (FAK, paxillin), force transduction layer (talin, vinculin), and actin regulatory layer (zyxin, VASP, β -actinin). This reveals for the first time a structural basis for FA function whereby a multilaminar architecture mediates the interdependent process of adhesion, signaling, force transduction, and actin cytoskeletal regulation.

The Effects of Sub-chronic Exposure to Inhaled Nickel Nanoparticles on the Cardiovascular System. G. Kang, P. Gillespie, L. C. Chen, Department of Environmental Medicine, New York University School of Medicine

This study was designed to determine if long-term exposure to inhaled nickel (Ni) nanoparticles (NPs) induced adverse effects on the cardiovascular system, resulting in exacerbated atherosclerosis in a sensitive mouse model. Male ApoE knockout mice were exposed either to filtered air or Ni NPs at $100 \mu\text{g}/\text{m}^3$ 10% of the current occupational guideline, for 5h/d, 5d/wk for either 1 week or 5 months. The analyses using bronchoalveolar lavage fluid (BALF) revealed significant oxidative stress and pulmonary inflammation at both time points. This data was consistent with gene expression analyses in the lung showing up-regulation of genes like heme-oxygenase-1 (Ho-1), interleukin-6 (Il-6) and monocyte chemoattractant protein-1 (Mcp-1). Those three genes were also up-regulated in the heart tissue after 5m of exposure, indicating systemic effects induced by inhaled Ni NPs. Furthermore, Mcp-1 was over-expressed in the aorta tissue, along with Cd68 and vascular cell adhesion molecule-1 (Vcam-1), after the 5-m exposure. This phenomenon coincides with increased plaque formation in the aortic arch, providing a molecular rationale for the exacerbated atherosclerosis. These results suggest that inhaled Ni NPs, at occupationally relevant levels, can induce significant chronic effects on the cardiovascular system, including exacerbation of atherosclerosis. Further studies will be needed to investigate potential mechanisms in which inhaled NPs could affect the cardiovascular system.

FOS Expression in Blood as a LDL-Independent Marker of Statin Treatment. J. G. Kang, H. J. Sung, S. I. Jawed, C.L. Breneman, Y. N. Rao, S. Sher, L. G. Biesecker, A. A. Quyyumi, V. Sachdev, P. M. Hwang, Translational Medicine Branch

We previously reported that the expression level of the *Finkel-Biskis-Jenkins Osteosarcoma (FOS)* gene, a transcription factor essential for monocyte to macrophage differentiation, was inversely correlated with statin therapy. We have now further examined the relationship between statin treatment and FOS mRNA levels expressed in the mononuclear cells of high risk patients as well as of those with established CHD. To show that mononuclear cell FOS mRNA levels are responsive to statin treatment, we treated a group of patients at high risk for CHD with statins for 3 months (10 mg atorvastatin equivalent) and observed a significant reduction in FOS mRNA levels in parallel with decreased LDL cholesterol. To further demonstrate the modulation of FOS expression by statin treatment, we examined FOS levels in a group of patients with known coronary artery disease who had their statin dosage titrated to similar LDL cholesterol levels according to clinical guidelines. In this cohort of patients, we observed an inverse relationship between FOS expression and statin dosage that was statistically significant at the highest statin dose (80 mg atorvastatin equivalent). Finally, we examined whether FOS expression in whole blood, and not just the mononuclear cell fraction, may also reflect statin activity. Using the PAXgene tube, we purified RNA from whole blood and observed an inverse relationship between FOS expression and statin dosage that was statistically significant between the low and high statin dosage groups. Our results show that FOS mRNA levels in blood may serve as a sensitive biological marker of statin treatment that is independent of LDL cholesterol and hsCRP levels

Characterization of Chondroitin Sulfate Using High Resolution Ion Trap Time-of-Flight Mass Spectrometry Y. Katagiri, Y. Wang*, P. Yu, J. H. Yi, D. A. Figge, F. Hays* H. M. Geller, Developmental Neurobiology Group, *Shimadzu Scientific Instruments

Glycosaminoglycans (GAGs) are a widely distributed, structurally diverse family of sulfated, unbranched polysaccharides expressed abundantly on the surface of cells and incorporated into the extracellular matrix. GAGs have emerged as important regulators of signaling pathways. One of the species of GAGs that is uniquely important in morphogenesis, cell division, and cartilage development is chondroitin sulfate (CS), the carbohydrate component of chondroitin sulfate proteoglycans. We have shown that CS GAGs play a pivotal role in axonal guidance and regeneration of the central nervous system. Due to the complex chemical structure and biological importance of CS, simple, sensitive, high resolution, and robust analytical methods are needed for the analysis of CS monosaccharides and disaccharides. [Method] CS monosaccharides and disaccharides were labeled with 2-aminobenzamide and separated on an Amide-HILIC column with ammonium formate as modifier to water acetonitrile based mobile phases. Simultaneous positive and negative LC/MS and LC/MSⁿ scans were collected using high resolution ion trap time-of-flight mass spectrometry. Accurate mass formula prediction with high resolution isotope matching was performed for each of the components identified as CS mono and disaccharides. MSⁿ spectra both in positive and negative mode were analyzed to verify the sulfation positions. [Result] Ten different CS mono and disaccharides were well separated by Amide-HILIC HPLC followed by MSⁿ analysis. All compound formulas were confirmed with less than 1 mDa mass error and high isotope pattern matching scores. Selective loss of sulfate group on some of the isomers revealed positional bond weakness of sulfation at 4-position compared to 6-position, subsequently distinguishing such isomers. Accurate mass of MSⁿ spectra also revealed fragment ion differences between isomers with identical masses. [Conclusion] We have developed a new method to detect small changes in CS GAGs derived from biological samples.

Human PDE3a: a Component of a Molecular Scaffold That May Integrate Myocardial Cyclic Amp and Calcium Atpase Signaling. F. A. Khan¹, W. Shen¹, J. Krall², F. Vandeput², E. Degerman³, M. Movsesian², V. Manganiello¹, ¹Translational Medicine Branch, ²University of Utah, Salt Lake City, UT, ³University of Lund, Sweden

Differential compartmentalization of signaling molecules in tissues and cells is recognized as an important mechanism for regulating the specificity of signal transduction pathways. Subcellular localization of PDE3 in human heart was determined by immunohistochemistry and sucrose gradient centrifugation. Different staining patterns for PDE3A and PDE3B were detected in myocardial membranes. PDE3A localized to sarcomere Z-bands and co-localized with desmin, AKAP18 and SERCA2, while PDE3B co-localized with mitochondrial proteins (COX4, ATP synthase, Cyt-C). In failing human myocardium, approximately 30% of the total membrane PDE3 activity is associated with plasma membrane/caveolae (PM) and 70% with internal membrane (Golgi/ sarcoplasmic reticulum) fractions. PDE3A3 is the dominant PDE3A isoform in PM/caveolae fractions, while PDE3B and all isoforms of PDE3A (A1-3) are present in internal membrane fractions. PM/caveolae fractions are highly enriched in β 2-ARs, β 3-ARs, $G\alpha(i)$, adenylyl cyclase 5/6, and caveolin-1, but not β 1-ARs or $G\alpha(s)$. On gel filtration chromatography, PDE3 activity in solubilized membrane fractions was partitioned between distinct

high molecular weight (HMW) and low molecular weight (LMW) peaks. The HMW peaks, which likely contain multiprotein complexes, contained PDE3A1, PDE3A2, and PDE3B, as well as several proteins involved in β -adrenergic receptor-mediated signaling, including β 1-AR, β 2-AR, AC5/6, PKA-RII, caveolin-1, and most of the AKAPs that we tested. PDE3 activity in cytosolic fractions consisted primarily of PDE3A2 and PDE3A3, and eluted only in LMW fractions. PDE3A was absorbed by and eluted from Rp-8-AHA-cAMP-agarose affinity gels. Coimmunoprecipitation of PDE3A and SERCA from solubilized myocardial membranes was confirmed by Western blots. Inhibition of PDE3 (in the presence of 0.3 μ M cAMP) stimulated microsomal Ca-ATPase (SERCA) activity, presumably by increasing phospholamban phosphorylation. These results indicate that PDE3 isoforms in myocardial membranes are likely to incorporate into multiprotein complexes; one such complex might contain PDE3A and SERCA2, suggesting a key role for PDE3A in the regulation of Ca²⁺ cycling in sarcoplasmic reticulum.

S-nitrosylation Exerts Cardioprotection During Ischemia-Reperfusion Injury by Reducing Cysteine Oxidation. M. J. Kohr^{1,2}, J. Sun¹, C. Steenbergen², E. Murphy¹, ¹Translational Medicine Branch, ²Department of Pathology, Johns Hopkins University, Baltimore, MD

S-nitrosylation (SNO) is greatly increased following myocardial ischemic preconditioning (IPC) and it has been proposed that SNO may provide cardioprotection, in part, by reducing cysteine oxidation during ischemia-reperfusion (IR) injury. To test this hypothesis, we utilized two-dimensional fluorescence difference gel electrophoresis (2D DIGE) with mass spectrometry to concomitantly examine SNO and oxidation in a model of IR injury. A modified biotin switch was performed using homogenates from hearts subjected to IR (60' perfusion, 20' ischemia, 5' reperfusion) and IPC-IR (20' perfusion, 4 cycles IPC, 20' ischemia, 5' reperfusion). Each sample was divided into two equal aliquots in order to analyze SNO and oxidation. After blocking free thiols with N-ethylmaleimide (NEM), SNO thiols were reduced with ascorbic acid (AA) and labeled with DyLight maleimide for detection. For oxidation, free and AA-reduced thiols were blocked with NEM, and the remaining oxidized thiols were reduced with dithiothreitol (DTT) and labeled. Four samples (IR_{SNO}, IR_{Ox}, IR-IPC_{SNO}, IR-IPC_{Ox}), each labeled with a unique DyLight maleimide fluor, were then combined and run via 2D DIGE. There was an increase in SNO proteins in IPC-IR compared to IR, including GAPDH, electron transfer flavoprotein, creatine kinase, α -enolase, α -ketoglutarate dehydrogenase (α -KGDH), and malate dehydrogenase. Consistent with the hypothesis, the majority of these SNO proteins showed decreased oxidation. Interestingly, α -KGDH produced 50% less H₂O₂ when pretreated with the nitrosylating agent S-nitrosoglutathione (GSNO) in an *in vitro* assay using purified enzyme. These results suggest that SNO yields cardioprotective effects by reducing the oxidation of cysteine residues partly through decreased H₂O₂ production.

Proteomic Analysis of Myosin II-mediated Focal Adhesion Maturation. J. Kuo¹, X. Han², J. Yates², C. M. Waterman¹, ¹Cell Biology and Physiology Center, ²Cell Biology, Scripps Research Institute, La Jolla CA

Focal adhesions (FA) are plasma-membrane associated macromolecular assemblies that serve to physically connect cells to, as well as transduce signals to and from, the surrounding

extracellular matrix (ECM). FA play a crucial role in the control of tissue structure and morphogenesis as well as cell motility. It is well established that FA undergo a tension-induced “maturation” process in which their size increases. Indeed, high cellular tension induced by myosin II activity promotes FA growth and maturation, while reduced cellular tension promotes formation of small, immature FA. The protein compositional changes that accompany maturation of FA are thought to be critical to modulating signals transduced from the ECM that regulate cell growth and differentiation. To determine how FA protein composition changes during FA maturation, we developed a systematic method to isolate FA from human fibroblasts in native morphology, identify their protein composition by Mud-pit LC-MS proteomics, and validate the presence of specific proteins in FA by a series of stringent criteria. We performed this method to determine how FA-associated proteins respond to myosin II activity to form FA structure by comparing the proteomic profiles of FA in the presence and absence of the myosin II inhibitor blebbistatin. The resulting data explores the relative abundance of FA components in FA changes appropriately during myosin II contraction-triggered maturation process. The appropriate FA assemblies transduce signals to regulate FA dynamics and cytoskeleton organization due to the biological functions of individual protein in FA. In addition, from the proteomic results, β -PIX, a Rac GEF, was found to be recruited to FA in blebbistatin-treated cells. We thus demonstrated that β -PIX was recruited to immature FA to maintain FA in immature state and promote Rac1 activation and lamellipodia formation. During myosin II contraction-triggered FA maturation, the abundance of β -PIX in FA decreased simultaneously. This study provides an insight into how myosin II contraction triggers biological responses through modulating FA composition.

Estrogen Reduces Ischemia-reperfusion (I/R) Injury via Pi3-kinase and Phosphorylation of Mitochondrial Dehydrogenases.

C. Lagranha¹, A. Deschamps¹, A. Aponte¹, C. Steenbergen², E. Murphy¹, Translational Medicine Branch¹, Johns Hopkins University²

Although pre-menopausal females have a lower risk for cardiovascular disease, the mechanism(s) are poorly understood. We tested the hypothesis that cardioprotection in females is mediated by altered mitochondrial protein levels and/or post-translational modifications. Using both an *in vivo* and an isolated heart model of I/R, we found that females had less injury than males, also males treated with estradiol had less injury than female ovariectomized. Using proteomic methods we found that female hearts had increased phosphorylation and activity of aldehyde dehydrogenase-2 (ALDH2), an enzyme involved in detoxification of ROS generated aldehyde adducts, and that an activator of ALDH2 reduced I/R injury in males but had no significant effect on I/R injury in females. Wortmannin, an inhibitor of PI3K, blocked both the protection and the increased phosphorylation of ALDH2 in females, but had no effect in males. Furthermore, we found an increase in phosphorylation of α -ketoglutarate dehydrogenase (α KGDH) in female hearts. α KGDH can be a major source of ROS generation. We found decreased ROS generation in permeabilized female mitochondria given α KGDH substrates, suggesting that increased phosphorylation of α KGDH might reduce ROS generation by α KGDH. In support of this hypothesis, we found that PKC dependent phosphorylation of purified α KGDH reduced ROS generation. Additionally, myocytes from female hearts had less ROS generation following I/R than males and the addition of wortmannin increased ROS generation in females to the same

levels as observed in males. These data suggest that post-translational modifications can modify ROS handling and play an important role in female cardioprotection.

Sunitinib Selectively Attenuates Airway Hyperreactivity, but not Airway Inflammation, in a Murine Model of Allergic Asthma.

J. K. Lam¹, H. C. Bhakta¹, J. Zhang¹, K. Fredriksson¹, M. Yu¹, X. Yao¹, K.J. Keeran², G. J. Zywicke², Z. X. Yu³, S. J. Levine¹, ¹Pulmonary and Vascular Medicine Branch, ²Laboratory of Animal Medicine and Surgery, and ³Pathology Core Facility

Multi-receptor tyrosine kinase inhibitors, such as imatinib, have been identified as potential therapeutic agents for asthma. Sunitinib is an oral, multi-target receptor tyrosine kinase inhibitor of c-Kit, PDGFR, VEGFR, CSF-1R, and Flt3 that is in clinical use for treatment of solid malignancies. Induction and treatment models of house dust mite (HDM)-induced allergic asthma were used to assess whether sunitinib can modulate airway inflammation, remodeling and hyperreactivity, which are the key pathogenic manifestations of asthma. In the induction model, asthma was induced in BALB/cJ mice by daily nasal administration of HDM, five days per week for three weeks, with concurrent sunitinib administration by oral gavage. In the treatment model, asthma was induced for six weeks, while sunitinib treatment was administered during weeks 4 through 6. Sunitinib administration significantly attenuated airway hyperreactivity (AHR) in HDM-challenged mice in both the induction and treatment models of allergic asthma. In contrast, sunitinib did not significantly inhibit HDM-induced increases in lung inflammation or lung mRNA levels of genes associated with Th2 mediated inflammation (*IL-4* and *IL-13*) or goblet cell hyperplasia (*Muc5AC*, *Clca3*). Sunitinib did not down-regulate IgE production, but instead increased serum IgE levels in the induction model. These results show that sunitinib selectively attenuates airway hyperreactivity in a murine model of HDM-induced allergic asthma. Furthermore, this demonstrates that sunitinib dissociates AHR from airway inflammatory responses, which is consistent with the conclusion that distinct pathways mediate these cardinal manifestations of asthma.

A Computational Investigation of the Nitrogen-Boron Interaction in *o*-(*N,N*-Dialkylaminomethyl)arylboronate Systems.

J. D. Larkin, J. S. Fossey, T. D. James, C. W. Bock, and B. R. Brooks, Laboratory of Computational Biology/Computational Biophysics Section

o-(*N,N*-dialkylaminomethyl)arylboronate systems are an important class of compounds in diol-sensor development. We report results from a computational investigation of fourteen *o*-(*N,N*-dialkylaminomethyl)arylboronates using second-order Møller-Plesset (MP2) perturbation theory. Geometry optimizations were performed at the MP2/cc-pVDZ level and followed by single-point calculations at the MP2/aug-cc-pVDZ(cc-pVTZ) levels. These results are compared to those from density functional theory (DFT) at the PBE1PBE(PBE1PBE-D)/6-311++G(*d,p*)(aug-cc-pVDZ) levels, as well as to experiment. Results from continuum PCM and CPCM solvation models were employed to assess the effects of a bulk aqueous environment. Although the behaviour of *o*-(*N,N*-dialkylaminomethyl) free acid and ester proved to be complicated, we were able to extract some important trends from our calculations: 1) for the free acids the intramolecular hydrogen-bonded B-O-H \cdots N seven-membered ring conformers **12** and **16** are found to be slightly *lower* in

energy than the dative-bonded N:B five-membered ring conformers **10** and **14** while conformers **13** and **17**, with no direct boron-nitrogen interaction, are significantly higher in energy than **12** and **16**; 2) for the esters where no intramolecular B-O-H...N bonded form is possible, the N:B conformers **18** and **21** are significantly lower in energy than the no-interaction forms **20** and **23**; 3) H₂O insertion reactions into the N:B structures **10**, **14**, **18**, and **21** leading to the seven-membered intermolecular hydrogen-bonded B...OH₂...N ring structures **11**, **15**, **19**, and **22** are all energetically favorable.

Progress Towards the Spatial and Temporal Modulation of HSPG Function in Zebrafish.

D. Lawrence, A. Srinivasan, K. Kramer, Laboratory of Developmental Glycobiology

Heparan sulfate proteoglycans (HSPGs) are a family of proteins characterized by their covalently attached, highly negatively charged heparan sulfate (HS) sugar side chains. Found at the cell surface and throughout the extracellular matrix, HSPGs mediate many important physiological processes including signal transduction, metabolism, and transport. During embryogenesis, HSPGs are critical to proper neuronal, heart and vascular tissue development, though the mechanisms by which they influence these processes are not well understood. Using morpholino gene knockdown techniques, we have observed intriguing early phenotypes in HSPG deficient zebrafish embryos, including defects in heart and vascular development. Such early phenotypes unfortunately mask the role of HSPGs in later stages of development. Moreover, morpholino knockdown experiments are unable to target specific tissue types. To surpass these limitations we have designed transgenic systems capable of tissue specific, small-molecule inducible transgene expression in order to achieve reliable spatial and temporal modulation of HSPG function.

Simultaneous Myocardial Perfusion and Strain Imaging with Displacement- encoded MRI.

Y. Le, K. Peter, J.Taylor, E. Bennett, K. Lucas, C. Chef'd'Hotel, C. H. Lorenz, P. Croisille, H. Wen, Translational Medicine Branch

The purpose of this study is to measure myocardial strain and perfusion simultaneously during the intravenous infusion of Gd-DTPA using a displacement-encoded MRI pulse sequence (DENSE). This method facilitates the correlation of function and perfusion. It should be especially useful for time-limited MR exams. The pulse sequence was tested in an acute coronary occlusion swine protocol (Yorkshire pigs, n = 9) and in an IRB approved ST-elevation myocardial infarction (STEMI) patient study (n = 11). Single-shot displacement-encoded images in 2-3 myocardial slices were repeatedly acquired using a single shot pulse sequence for 3 to 4 minutes, which covers a bolus infusion of Gd. The magnitudes of the images were T₁ weighted and provided quantitative measures of perfusion, while the phase maps yielded strain measurements. Post-processing included elastic registration of the images and quantitative mapping of perfusion, contrast washout time constant and circumferential strain. The animal protocol consists of LAD or LCX occlusion. MRI and microsphere data were acquired and compared. In the patient data, the perfusion-function and perfusion-wash out rate correlations were performed. In the swine protocol, segmental perfusion measurements were validated against microsphere reference standard with a linear regression (slope 0.986, R² = 0.765, Bland-Altman standard deviation = 0.15 ml/min/g). In the patient study the scan success rate was 76%. Short-term contrast washout rate and perfusion are highly corre-

lated (R²=0.72), and the pixel-wise relationship between circumferential strain and perfusion was better described with a sigmoidal Hill curve than linear functions. This study shows that a displacement-encoded pulse sequence is able to assess myocardial strain and perfusion simultaneously. Ventricular function and perfusion are two important indexes in the evaluation of heart disease so this method is able to measure both simultaneously and helps to shorten the time of a cardiac MR exam.

The Essential Autophagy Gene Atg7 Coordinates Cell Cycle Withdrawal and Survival with Nutrient Status.

I. H Lee, L. Cao, Y. Kawai, M. M. Fergusson, I. Rovira, A. J.R. Bishop, N. Motoyama and T.Finkel, Molecular Biology Section

Withdrawal of nutrients triggers an exit from the cell cycle, the induction of autophagy and ultimately when prolonged, the activation of cell death pathways. The relationship, if any, between these events has not been fully characterized. Here we demonstrate that cells lacking the essential autophagy gene Atg7 fail to properly exit the cell cycle following nutrient withdrawal or upon reaching confluency. This inability to arrest is accompanied by a specific lack of induction of the p53 transcriptional target and cell cycle inhibitor p21^{CDKN1A}. We further demonstrate that Atg7 and p53 form a nutrient sensitive complex that involves the carboxy-terminus of Atg7 and the tetramerization domain of p53. In an Atg7 dependent fashion, starvation is shown to increase p53 multimer formation and transcriptional activity. During metabolic stress conditions, Atg7 deficient cells fail to undergo cell cycle arrest and in turn, exhibit significantly increased levels of cell death through a pathway involving Chk2 and p53 activation. The physiological importance of this pathway is underscored by the observation that deletion of Chk2 can prolong the overall post-natal survival of Atg7 deficient mice. These results demonstrate that Atg7 represents a molecular interface between the cell cycle and autophagy machinery and serves as an important overall regulator of p53 activity under conditions of metabolic stress.

The Calcium Channel MCOLN3 Regulates Endosomal Acidification.

B. Lelouvier and R. Puertollano. Laboratory of Cell Biology

The cation channel mucolipin 3 (MCOLN3 or TRPML3) is one of the three members of the mucolipin family. A punctual mutation in the mouse gene of MCOLN3 results in the varitint-waddler phenotype characterized by auditory and vestibular defects as well as pigmentation abnormalities.

Recent observations show a strong involvement of the mucolipin family in protein trafficking and recycling along the endo-lysosomal pathway. MCOLN3, suspected to play a role in melanosome trafficking, was recently shown to cause a dramatic alteration in the endosomal pathway when misexpressed. Indeed modification of the expression of MCOLN3 induces enlargement of HRS positive compartments, affects autophagosome maturation, and impairs degradation of epidermal growth factor (EGF) and EGF receptor. Calcium and pH play a crucial role in cargo trafficking, membrane fusion and in the biogenesis and regulation of the endosomal machinery. Because MCOLN3 is a Calcium-permeable channel regulated by pH, we hypothesize that MCOLN3 could couple pH and Calcium concentration along the endosomal pathway.

To elucidate the function of MCOLN3 and the consequences of its misexpression, we analyzed the outcomes of its over-expression, depletion or mutation on endosomal pH and

Calcium concentration. *In vitro* endosomal fusion assay were also performed to gain additional information about the function of MCOLN3 and the acidification and calcium regulation of the endosomal pathway.

Membrane Diffusion of Tethered Dppc and Tethered Pip3-bound Protein Systems. M. G. Lerner and R. W. Pastor, Laboratory of Computational Biology

We have used molecular dynamics simulations to investigate the diffusion of tethered proteins in lipid bilayers. Coarse-grained (CG) models of DPPC dimers were simulated in a DPPC bilayer with the MARTINI model, and single-lipid diffusion constants compared to those obtained for dimers at various tether lengths. The ratio of diffusion constants matches well with both experimental results and theoretical predictions of a simple bead model. A full theoretical model of the translational and rotational diffusion of tethered dimers was developed at the Saffman-Delbruck level, and results are compared to the simple bead model. CG models of pleckstrin homology domain (PH) bound to a lipid with a PIP₃ (phosphatidylinositol (3,4,5)-trisphosphate) head group, along with simpler models of DPPC-bound proteins, were then constructed and compared to the monomer and tethered dimer cases.

Cxcl12-cxcr4 Signal Regulates Nerve-mediated Arterial Branching in the Developing Limb Skin. W. L. Li, H. Zang, K. Soneji, Y. Mukoyama, Laboratory of Developmental Biology

The blood vessel branching network develops from a primary capillary network, which undergoes intensive vascular remodeling during angiogenesis. Proper vascularization of organs and tissues requires not only angiogenesis and arteriovenous differentiation, but also appropriate patterning of the vascular network. We have previously discovered that nerve provides a template that determine arterial vessels branching pattern and nerve-derived VEGF-A is required for artery differentiation but not for nerve-vessel alignment in embryonic mouse limb skin. But the alignment and patterning process may require nerve-derived signals other than VEGF-A is still unclear. Using gene expression profiling, we show that C-X-C motif chemokine ligand (CXCL) 12 is highly expressed by migrating Schwann cells in peripheral nerves and a patchy expression of its functional receptor, CXCR4, is seen in capillary-like endothelial cells. Mutant embryonic limb skin of CXCL12 and CXCR4 as well as in endothelial cell-specific deletion of CXCR4 mutant embryos show the disruption of nerve-artery alignment. Furthermore, *in vitro*, endothelial cell migration mediated by the supernatant from cultured embryonic dorsal root ganglia (DRG) is inhibited by a neutralized antibody and small compound inhibitor of CXCR4. Our results indicate that CXCL12 is the nerve-derived chemoattractive cytokine, which controls the proper nerve-artery network formation through endothelial CXCR4 in the developing limb skin.

Characterization of Zebrafish Glycoproteins 7a and 7b as Heparan Sulfate Proteoglycans, and Specifically as Glypicans. C. D. Liepmann, K. Kramer, Genetics and Development Biology Center

Proteoglycans are ubiquitously expressed extracellular macromolecules that modify multiple cell signaling pathways. Characterized by heavy glycosylation, proteoglycans have at least one glycosaminoglycan (GAG) chain attached to a core protein. The specific type of proteoglycan is determined by the identity of the GAG chain; the two families of proteoglycans are distin-

guished by their attachment to the cell surface: syndecans are transmembrane proteins whereas glypicans are anchored to the membrane by glycosylphosphatidylinositol (GPI). While there are six vertebrate glypicans characterized, the genome wide duplication during the evolution of bony fish suggests that twice as many glypicans could exist in zebrafish. Through analysis of zebrafish and evolutionarily-related organisms' genomes, we discovered two novel glypicans with homologous core protein structures in zebrafish: zGPC7a and 7b. To verify the classification of zGPC7a and 7b we assessed their glycosylation by transfecting two distinct cell lines and performing gel electrophoresis. We were able to classify 7a and 7b as HSPGs by detecting the characteristic amino acid stubs that anchor HS to the core proteins. The evidence presented supports the classification of 7a and 7b as heparan sulfate proteoglycans. Further experiments will analyze the effect of these novel glypicans on cell signaling.

Copper(I) and Copper(II) Binding to α -Synuclein. H. R. Lucas and J. C. Lee, Laboratory of Molecular Biophysics

α -Synuclein (α -syn), an amyloidogenic protein implicated in Parkinson's disease, binds copper(II) ion with high affinity based on *in vitro* studies. Tryptophan fluorescence measurements (F4W and F4W/H50S) indicate that copper(II) binding is conserved within the first four α -syn residues MDV(F/W) and that the N-terminal amine is the anchoring ligand. Using site-directed mutagenesis (D2N/F4W and D2E/F4W), we also probed the nature of the copper(II)-D2 interaction showing that D2 is an unlikely copper(II) ligand. Complementary data on analogous N-terminal peptide mutants has also been collected and further examined by circular dichroism analyses. Structural information has been further gained through X-ray absorption spectroscopy on copper(II) bound α -syn (wild-type) in both the soluble and fibrillar forms as well as in the presence and absence of dioxygen. New insights on the copper(I) coordination environment will also be discussed as well as potential deleterious copper-dioxygen chemistry, such as Met oxidation and/or Tyr crosslinking. Additionally, we examined the ability of membrane-bound α -syn to bind copper(II/I) ion(s) and how copper binding may modulate protein-membrane interactions since α -syn is known to localize near synaptic vesicles *in vivo*.

A Role for Nonmuscle Myosin II-C in Cardiac Myocyte Karyokinesis and in Maintaining Integrity of Cardiac Myocytes X. Ma, S. S. Jana, S. Kawamoto, M. A. Conti, and R. S. Adelstein, Laboratory of Molecular Cardiology

To understand the *in vivo* function of nonmuscle myosin (NM) II-C, we generated two lines of knockout mice, one in which only the alternatively spliced exon, C1 is ablated and a second with total ablation of NM II-C. Both lines of mice survive to adulthood and show no obvious defects compared to wild type littermates. However, when NM II-C is ablated in mice which express only 12% of wild-type amounts of NM II-B, the resulting mice show a further increase in cardiac myocyte enlargement. In addition, these hearts develop interstitial fibrosis associated with diffuse N-cadherin and β -catenin localization at the intercalated discs. When both NM II-C and II-B are ablated the B-C/B-C cardiac myocytes show major defects in karyokinesis. Over 90% of B-C/B-C myocytes demonstrate defects in chromatid segregation and mitotic spindle formation accompanied by increased stability of the microtubules. This requirement for NM II in karyokinesis is further demonstrated in cultured HL-1 atrial myocytes using siRNA knockdown of NM II or treatment with the myosin inhibitor

blebbistatin. Our study shows for the first time that NM II-C together with NM II-B is involved in maintaining the integrity of the cardiac myocytes as well as in regulating cardiac myocyte karyokinesis.

Distinct Sorting Determinants Guide the Trafficking Itinerary of the New Clathrin-independent Endocytic Cargo Proteins CD44 and CD147. L. Maldonado-Báez, N. B. Cole, C. A. Eyster and J. G. Donaldson, Laboratory of Cell Biology

Clathrin-independent endocytosis (CIE) mediates the internalization of transmembrane proteins devoid of clathrin- or clathrin-adaptor targeting sequences. In HeLa cells, CIE cargo, such as the major histocompatibility complex Class I protein (MHCI) and the IL2 receptor α -subunit (Tac), enters the cell in non-clathrin vesicles that later fuse with EEA1- and transferrin-positive endosomes. Eventually, the cargo is either degraded in late endosomes or recycled back to the plasma membrane through recycling tubules. Although, this pathway is the preferred route for many CIE cargoes, we have identified two new CIE cargo proteins, CD44 and CD147 that differ in their trafficking from MHCI/Tac once captured in CIE vesicles. CD44 and CD147 do not travel to EEA1-endosomes. Instead, they are routed directly to the recycling tubes. These observations suggest that sorting mechanisms may guide the trafficking of CIE cargo. To identify potential sorting determinants in the sequence of CD44 and CD147, we created chimeras consisting of the luminal domain of Tac and CD44 or CD147 sequences (Tac-CD44-CD44 or Tac-CD147-CD147) and examined their trafficking after endocytosis. In contrast to the trafficking of Tac, the chimeras by-passed EEA1-endosomes, evaded late endosomes and accumulated in recycling tubules. The traffic of the chimeras resembled that of CD44 and CD147, indicating that these proteins contain sequence information that alters the trafficking of Tac. Currently, we are assessing the role these sequences play in the sorting of CIE cargo. Our data suggests that distinct sorting determinants guide the itinerary and final destination of different groups of CIE cargo molecules.

Levels of Placental Growth Factor and Endothelin-1 in Adults with Sickle Cell Disease are Linked to Markers of Hemolysis, Inflammation, Iron Overload and Pulmonary Hypertension. L. Mendelsohn, X. Wang, D. Allen, C. Minniti, J. G. Taylor VI, and G. J. Kato, Pulmonary and Vascular Medicine Branch

Pulmonary hypertension (PH) is one of the most clinically significant complications of sickle cell disease (SCD). We have recently shown that placental growth factor (PIGF), an angiogenic factor produced by erythroid cells, promotes release of the potent endogenous vasoconstrictor endothelin 1 (ET-1). ET-1 is a potent vasoconstrictor that is associated with PH in other patient populations. ET-1 has been reported to be high in patients with SCD, rising further during vaso-occlusive crisis and acute chest syndrome. We measured the levels of PIGF and ET-1 in plasma samples from 123 adults with SCD, and evaluated statistical correlations with standard clinical laboratory markers. Consistent with PIGF induction of ET-1, we find in plasma specimens from adults with SCD that the two are significantly correlated ($p < 0.01$), consistent with previous results outside of SCD. Interestingly, our results have shown that both PIGF and ET-1 levels are correlated with tricuspid regurgitant jet velocity (TRV) ($p < 0.001$ for both), a Doppler echocardiography marker of PH. Furthermore, ET-1 also correlates with a second marker of PH, plasma NT-proBNP ($p < 0.05$). These results implicate the PIGF-ET-1 pathway

in PH in SCD. In additional support of this model, the level of PIGF is linked with several markers of hemolysis, iron overload and hepatic dysfunction that are reproducibly linked to PH in SCD: low hemoglobin ($p < 0.01$) and transferrin ($p < 0.001$); and high serum lactate dehydrogenase ($p < 0.05$), direct bilirubin ($p < 0.05$), ferritin ($p < 0.001$), and transferrin saturation ($p < 0.01$). Interestingly, PIGF also is correlated to leukocyte count ($p < 0.05$) and C-reactive protein ($p < 0.01$), which in the general population are inflammatory markers that contribute to the risk of atherosclerosis, another proliferative vasculopathy with mechanistic parallels to PH in patients with SCD. Unexpectedly, PIGF and ET-1 are induced by hydroxyurea treatment, potentially explaining the inconsistency of TRV reduction by the drug. ET-1 appears to be a mediator of elevated pulmonary artery pressures as estimated by echocardiography in patients with SCD. Its expression is related in part to PIGF, the associations of which match the established profile of the average SCD patient with PH. This finding lends additional support to our recently published case series preliminarily suggesting clinical efficacy of the ET-1 receptor antagonists bosentan and ambrisentan in SCD adults with PH. The potential therapeutic benefit of ET-1 receptor antagonists merits further clinical trials.

BCL2A1 is a Survival and Immortalization Factor for Primitive Hematopoietic Cells. J. Y. Métais, T. Winkler, R. T. Calado, and C. E. Dunbar. Hematology Branch

We recently reported the development of an acute myeloid leukemia in a rhesus macaque transplanted with autologous CD34+ cells transduced with a murine stem cell virus-derived replication defective retrovirus vector expressing only marker genes under control of the strong MCSV LTR. This tumor was the first hematopoietic malignancy reported in a recipient of primitive cells transduced with a replication-incompetent vector containing only marker genes, and suggested that BCL2A1 could have potent effects on hematopoiesis. To further investigate the impact of the BCL2A1 gene product on normal and malignant hematopoiesis, we cloned the murine and human HA-tagged BCL2A1 cDNAs into lentiviral vectors and transduced the murine BaF3 hematopoietic cell line as a model to study the impact of expression of these proteins in vitro. The proliferation and survival of the BaF3 cells is dependant on IL-3. Upon removal of IL-3 from the media, BaF3 cells underwent an arrest in the G1 phase of the cycle. Untransduced cells or cells transduced with the empty lentiviral vector were 45% apoptotic, but this fraction decreased to 30 and 15% respectively with the cells transduced with murine and human BCL2A1. Similar results were obtained in murine 32Dcl3 cells and human UT7/Epo-S1 cells, two cell lines that are respectively dependant on IL-3 and erythropoietin. In order to study the in vivo impact of BCL2A1 on hematopoiesis, C57/bl6 (Ly5.2) mice have been transplanted with primary bone marrow cells (from C57/bl6 (Ly5.1)) transduced with the BCL2A1 and control vectors, and have been followed for in vivo expansion of transduced clones and development of leukemia. The mice cohort consisted of 5 MOCK controls, 15 control vector expressing GFP only, and 15 murine BCL2A1. We frequently checked the blood counts and analyzed the lineage of blood by FACS. Mice transplanted with marrow cells transduced with the BCL2A1 vector had higher overall marking levels in the blood compared to the vector control (80% vs. 10% cells GFP+ respectively). Interestingly, preliminary histopathology revealed that 7 mice from the BCL2A1 group, but

none of the control groups developed a further to be classified very poorly differentiated hematologic malignancy consisting of circulating blasts, splenomegaly, and lymphadenopathy. Cells responsible for this fatal disease were not stained by markers used for FACS analysis or by immunohistochemistry. The median overall survival was significantly different for mice treated with the vector (420 days) and mice treated with BCL2A1 (328 days). The median disease free survival was more striking as the median could not be defined for the vector while it was of 397 days for BCL2A1. Two hundred and fifty three days after the transplantation we selected 3 primary mice from BCL2A1 and vector control groups to perform secondary transplant. Less than a month after reinfusion all BCL2A1 mice developed a disease characterized by high white blood cell counts with a majority of undifferentiated cells, as well as splenomegaly, and hepatomegaly. These results were repeated in a second set of secondary transplant carried out 289 days after transplantation confirming that the disease is transplantable. The preliminary histopathology findings showed a similar phenotype to the lymphatic malignancy seen in the primary mice. In conclusion, we have confirmed the anti-apoptotic role of BCL2A1, and we are now investigating its role in hematopoiesis and leukemogenesis. We are investigating the exact phenotype of the disease seen in primary and secondary mice in order to clarify the role of BCL2A1 as a survival factor.

MSCALE: A Framework for Multiscale Molecular Simulations in CHARMM. B. T. Miller, H. L. Woodcock, M. Hodosek, A. Okur, J. D. Larkin, and B. R. Brooks, Laboratory of Computational Biology

A new CHARMM command, MSCALE, has been implemented to support arbitrary types of multi-scale simulations. The MSCALE command allows CHARMM to call external programs (including other copies of itself) that have been designed to interface with it. The external program is then used to calculate the energy or forces on all or part of the system. This poster will describe three different cases where MSCALE enhances the capabilities of CHARMM: (1) in setting up QM/MM and QM/SE simulations using ONIOM, (2) in conducting free energy perturbation calculations where the force field itself is modified, and (3) in performing normal mode analysis of multiscale models. Results of each of these three types of cases are presented. Furthermore, CHARMM has been interfaced with external programs such as AMBER allowing for additional possible simulation types. Finally, this poster will discuss the integration of MSCALE with the CHARMMing web GUI.

Linkage of Leg Ulcers to Pulmonary Hypertension in SCD. C. P. Minniti¹, M. Hildesheim¹, D. Allen¹, O. Castro², and G. J. Kato¹, ¹Pulmonary and Vascular Medicine Branch, ²Center for Sickle Cell Disease, Howard University, Washington, DC

Non-healing leg ulcers are a debilitating complication of sickle cell disease (SCD), affecting 8 to 50% of patients. We evaluated the clinical and laboratory characteristics of patients with SCD and a history of leg ulceration. **Methods:** Three hundred ninety six SCD adult patients were enrolled in a NHLBI-approved protocol and screened for pulmonary hypertension. We collected a detailed past medical history, and a comprehensive set of laboratory tests. Comparisons between patients with and without leg ulcers were made using Wilcoxon rank sum tests. Associations between categorical variables and leg ulcer in the two groups were tested using the chi-square test of independence. Kaplan Meier survival curves were generated to examine differ-

ences in mortality. Patients were followed for an average of 43.5 months (25th and 75th percentiles: 22 and 65 months). **Results:** Eighteen % of all subjects had a history of leg ulceration (21.4% of SS and 9.6% of SC). Patients affected were older, had markers of severe hemolysis, high serum uric acid, alkaline phosphatase, NT-proBNP and lower serum albumin. They had a higher prevalence of elevated tricuspid regurgitation velocities, suggestive of pulmonary hypertension. They were more likely to have died by the time of analysis (21% vs. 9%, P=0.02), with a similar trend on Kaplan-Meier. History of pain, acute chest syndrome, stroke or priapism, were not associated with leg ulceration. Hydroxyurea or chronic transfusions did affect the incidence of leg ulcers. **Conclusions:** Risk factors for leg ulcers in SCD are: HbSS genotype, high hemolysis, serum uric acid, elevated TRV and NT-proBNP. SCD patients with leg ulcers have higher mortality. Our results do not support or disprove the use of chronic transfusions or hydroxyurea for leg ulcers.

A Drosophila Neuroigin Is Required for Synapse Formation and Function. B. Mozer¹, and D. Sandstrom², ¹Laboratory of Biochemical Genetics, NHLBI, ²Laboratory of Molecular Biology, NIMH

Neuroligins encode a family of cell adhesion molecules that possess synapse-inducing activity in vitro and are required for synaptic function in vivo. Rare loss of function mutations or copy number variations of human neuroigin genes are associated with autism, a neurodevelopmental disorder affecting children. Although the vertebrate neuroigin gene family has been the focus of extensive research, little is known about the function of these genes in a genetically tractable organism such as the fly. Bioinformatic analysis of the Drosophila genome revealed four genes sharing 35-40% identity to the extra cellular ligand-binding domain of vertebrate neuroligins. The four genes are likely functional homologues as they share many of the domain signatures common to neuroigin family members including a catalytically inactive esterase/lipase ligand binding domain, a single pass transmembrane domain and a cytoplasmic tail predicted to bind to PDZ domain containing proteins. We have analyzed the function of the fly neuroigin 2 gene, (*fnlg2*) in the larval neuromuscular junction, which serves as a model synapse. A *fnlg2* mutant is homozygous viable, but has a reduction of synapse size. Electrophysiological recordings revealed a corresponding functional defect in the mutant. Evoked junctional currents were significantly reduced in the mutant muscle in response to stimulation of the nerve while the frequency and amplitude of spontaneous miniature excitatory currents was normal. The neuro-anatomical and electrophysiological phenotypes of the *fnlg2* mutant could be rescued by an 80KB genomic construct containing the entire gene. However, neither neural-specific nor muscle-specific expression of a *fnlg2* cDNA transgene was sufficient to rescue the mutant defects. Over-expression of *fnlg2* in neurons in a wildtype genetic background did not result in an increase in synapse numbers suggesting that increased neuroigin expression is not sufficient for synapse formation. Immunostaining of larval body walls with antiserum that is specific for *fnlg2* protein revealed pre-synaptic localization in the nerve terminals and diffuse localization of the protein at the cell surface of the muscle. Synaptic growth in the fly is negatively regulated by protein degradation and positively regulated by electrical activity. In synaptic overgrowth mutants such as *highwire* (*hiw*) or *ether-a-go-go shaker* (*eag sh*) *fnlg2* protein failed to accumulate in the synapse. These

findings are consistent with a model for neuroligin in the maintenance and function of synapses rather than their growth.

Myosin-II Mediated Regulation of Microtubule Dynamics Controls Endothelial Cell Branching Morphogenesis. K. A. Myers¹, K. T. Applegate², R. S. Fischer¹, G. Danuser³, C. M. Waterman¹, ¹Laboratory of Cell and Tissue Morphodynamics, ²Laboratory for Computational Cell Biology, The Scripps Research Institute La Jolla, CA, ³Laboratory for Computational Cell Biology, Harvard Medical School, Boston, MA

Extracellular Matrix (ECM) dimensionality and stiffness regulate endothelial cell branching morphogenesis via mechanosensitive cell adhesion receptors that elicit bidirectional signals to and from the actomyosin cytoskeleton. Stiff ECMs promote myosin-II activity that inhibits cell branching, while compliant ECMs reduce myosin-II activity and promote branching. The microtubule (MT) cytoskeleton controls cell morphology through its structural and regulatory interactions with actomyosin. However the role of MT organization and dynamics in the cellular responses to ECM dimensionality and compliance remain poorly understood. To explore this role, we analyzed MT and actin organization in HUVEC cells on two different stiffnesses of 2D and 3D ECMs. We find that MT presence within branches is correlated with branch elongation, and that inhibition of MT dynamics inhibits branch elongation and directed cell motility. To determine how ECM affects MTs, we analyzed MT dynamics by tracking fluorescently-tagged MT plus ends using novel automated software. This revealed that ECM dimensionality and compliance mechanosensing work synergistically to promote a global increase in fast, dynamically unstable MT growth. Fast and dynamic MT growth excursions become slower and more persistent specifically within cell branches, where cell-ECM contacts are established and myosin-II contractility is locally enriched. Pharmacologic (global) inhibition of myosin-II contractility compromises the localized effects of both compliance and dimensionality, causing an increase in the fastest and most dynamically unstable MT growth excursions throughout the cell. Thus, we define a regulation scheme in which ECM-induced alterations of local myosin-II contractility regulates MT dynamics locally, in order to control cell branching morphogenesis.

Kinetic Characterization of Non-muscle Myosin IIB SH-HMM and HMM on Single Molecule Level with Optical Tweezers. A. Nagy¹, Y. Takagi¹, E. Homsher^{1,2}, D. K. T. Hong³, M. Kovács⁴ and J. R. Sellers¹, ¹Laboratory of Molecular Physiology, ²Department of Physiology, David Geffen School of Medicine at UCLA, ³ABT Software, Washington, D.C., ⁴Department of Biochemistry, Eötvös University

Non-muscle myosin IIB (NMIIB) is a cytoplasmic conventional myosin, which plays an important role in development of the brain and heart, and in directed growth cone motility by maintaining cortical tension in motile cells. It forms short bipolar filaments with ~14 myosin molecules on each side of the bare zone. NMIIB is a very slow myosin both in terms of actin-activated ATPase activity and actin translocation capability. Our previous studies showed that the NMIIB is a moderately high duty ratio (~20-25%) motor. The ADP release step (~0.35 s⁻¹), of NMIIB is only ~3 times faster than the rate-limiting phosphate release (0.13 ± 0.01 s⁻¹). Because of its slow ADP off-rate, acto-NMIIB has the highest ADP-affinity reported so far for the myosin superfamily (<0.15 μM). To examine the mechanics and kinetics of NMIIB at the single-molecule level we used a dual-beam optical tweezers to perform the "three-bead" assay. The surface-immobilized bead was

coated with recombinantly engineered single-headed heavy mero-myosin-like (NMIIB-SH-HMM) and HMM molecules. We measured the lifetimes of unitary actomyosin interactions and determined the actin-detachment kinetics with varying ATP concentrations. Results showed that at physiological ATP concentration (1 mM), the rate of detachment of acto-NMIIB-SH-HMM and HMM interactions were similar to the ADP release rate and steady-state ATPase rate reported from solution kinetic studies. Decreasing the ATP concentration to 1 μM did not alter this rate of detachment. Also, our results showed that the power-stroke of NMIIB-SH-HMM and HMM were ~8 nm. We will discuss our single-molecule results from the perspective of the essential cellular functions of NMIIB in cell locomotion, tension generation and maintenance.

Neuro-Vascular Interaction in Embryonic Heart Development. J. Nam, Y. Uchida, I. Onizuka, J. Hatch, and Y. Mukoyama, GDBC/LDB

Coronary vasculature has always been a major subject of interest for developmental biologist. In embryonic mouse, the heart is innervated by bilateral sympathetic cardiac nerves originating from the sympathetic ganglia, but the mechanisms that dictate the patterning of coronary vessels and cardiac nerves remain largely unknown.

While vascular networks in embryonic limb skin are guided by peripheral nerve innervations, a similar interaction could potentially exist between coronary vessels and cardiac sympathetic nerves. To explore this interaction, mouse embryonic hearts aged between E13.5 through E15.5, the period during which most vascular reformatting takes place, are analyzed.

In one mutant having a lack of cardiac axons, remodeled vascular pattern appears to be normal, suggesting the cardiac nerve is not prerequisite for vasculature remodeling.

Whereas in the other mutant, where the cardiac vasculature is disorganized, the patterning of cardiac nerve is also disrupted. These results suggest a possible vascular guidance of cardiac sympathetic axon during cardiac development.

An RNAi Screen of Microtubule-Regulatory Proteins Identifies MARK2/Par1 as an Effector of Rac1-mediated Microtubule Growth. Y. Nishimura¹, K. Applegate², G. Danuser³, C. Waterman¹, ¹Laboratory of Cell and Tissue Morphogenesis, ²Laboratory for Computational Cell Biology, The Scripps Research Institute, La Jolla, CA, ³Laboratory for Computational Cell Biology, Harvard Medical School, Boston, MA

Proper regulation of microtubule (MT) assembly dynamics is essential for directed cell migration. MT dynamics in migrating cells are spatially regulated by Rho GTPases. We have previously shown that activated Rac1 induces MT net growth by suppressing catastrophe and increasing growth velocity, and that Rac1 activity is required for polarized MT growth in the leading edge of migrating cells. We identified a necessary, but not sufficient, PAK kinase-mediated pathway downstream of Rac1 that promoted MT growth. Therefore, we hypothesized that additional factors promote MT net growth downstream of Rac1. To find these factors, we performed an RNAi screen in human U2OS osteosarcoma cells to determine if known MT-regulatory proteins were required for constitutively activated Rac1 promotion of MT growth. To analyze MT dynamics, we imaged fluorescent-tagged EB3, a MT plus-end binding protein that serves as a probe for the position of MT ends, and tracked the motion of EB3 comets in time-lapse movies using an automated computer program. Our

results indicate that depletions of several MT-binding proteins change the growth rate of MTs in activated Rac1-expressing cells. We have focused on MARK2, a MT affinity-regulating kinase homologous to the *C. elegans* polarity protein Par1, whose depletion reduces the number of elongated MTs in the leading edge of Rac1-activated cells. We are currently testing how MARK2 is involved in promoting MT growth downstream of Rac1 and its requirement in cell migration.

Neuronal Control of Lymphatic Vessel Development in the Immunoprivileged CNS. E. O'Donnell, K. Soneji, Y. Uchida, Y. Mukoyama, Cardiovascular Developmental Biology

The Central Nervous System (CNS) has been identified as an immunologically privileged organ based on the absence of conventional lymphatic vasculature. Besides the physical separation of the CNS from the systemic immune system, provided by the Blood Brain Barrier (BBB), scientists believe that signals from the CNS prevent lymphangiogenesis and thus maintain an immunoprivileged microenvironment. Our studies focus on understanding what factors from the spinal cord block lymphatic vessel development. Extensive fate mapping experiments show that lymphatic endothelial progenitors arise from venous endothelial cells (ECs). Although there are no LYVE-1+ lymphatic vessels in the embryonic spinal cord, CD34+ (and PECAM-1+) blood vessels do exist. We have found that supernatant from cultured embryonic spinal cords prevent the differentiation of ECs into lymphatic ECs. We have also shown that the spinal cord supernatant inhibits the migration of primary lymphatic, but not blood ECs. Now, we are studying what factors in the spinal cord supernatant prevent the differentiation and migration of lymphatic vasculature in the CNS. Several candidate factors, all expressed by the spinal cord, that have been shown to hinder differentiation of ECs into lymphatic ECs in vitro include VEGF-A, b-FGF, TGF-beta 1, 2, and 3. Our future studies will focus particularly on analyzing lymphangiogenesis in mutant mice that lack TGF-beta signaling. Understanding how signals from the CNS influence lymphatic development will have important implications on clinical diseases such as multiple sclerosis (MS), an immune-mediated disorder that occurs in the CNS.

Combining Conformational Space Annealing with Replica Exchange Method for Improved Conformational Search. A. Okur, J. Lee, B. Brooks, Laboratory of Computational Biology

Replica exchange molecular dynamics (REMD) has been successfully used to improve the conformational search for model peptides and small proteins. However for larger and more complicated systems the use of REMD is still computationally intensive since the number of replicas required increases with system size. Achieving convergence with systems with slow transition kinetics is also very difficult. Several methods have been proposed to overcome the size and convergence speed issues of REMD. One of these methods is called Reservoir Replica Exchange Method (R-REMD) where the conformational search and temperature equilibration are separated. This allows integrating computationally efficient search algorithms with replica exchange. The Conformational Space Annealing (CSA) method has been shown to be able to determine the global free energy of proteins efficiently and has been used in structure prediction successfully. CSA uses a genetic algorithm approach to perform the conformational search and determine the minimum energy structure. We have used conformations generated through CSA method and collected them in a reservoir. Replica exchange was then performed where the top

replica was seeded with the reservoir structures and fast convergence at every temperature is observed. We have tested this method with model peptides and significant improvement on efficiency was observed.

Coronary Smooth Muscle Sells Guide Sympathetic Axon Growth. I. Onitsuka, J. Nam, Y. Uchida, and Y. Mukoyama, Genetics and Developmental Biology Center

Sympathetic neurons innervate and control a wide range of targets including heart. A fundamental question is how subsets of neurons acquire molecular properties that guide axonal trajectories to appropriate target-organs. We have recently found that sympathetic neurons send initial axonal projections along the remodeled large coronary veins within the subepicardial tissue layer of the dorsal and lateral walls of the two ventricles in developing heart. In the epicardium-specific b-catenin mutants having disorganized coronary vasculature, proper sympathetic innervation fails to occur. Taken together, coronary vessels, especially remodeled large coronary veins, may secrete axon guidance cues that direct sympathetic axons into the heart.

Since remodeled coronary veins are covered by aSMA⁺ smooth muscle cells (SMCs), we first examined whether the epicardium-derived SMCs provide attractive guidance cues to sympathetic axons. For these experiments, we cocultured embryonic sympathetic ganglia (SG) explants with or without the epicardium-derived SMCs in a collagen gel support. Indeed, robust extension of neurites from SG explants when SMCs were cocultured in the collagen gel. Now we are extensively working on what factors derived from SMCs control sympathetic axon growth and guidance in the developing heart.

Myosin II Activity Regulates Vinculin Recruitment to Focal Adhesions Through Fak-mediated Paxillin Phosphorylation.

A. M. Pasapera¹, I. C. Schneider³, E. Rericha², D. D. Schlaepfer⁴, C. M. Waterman¹, ¹Cell Biology and Physiology Center, ²Institute for Research in Electronics and Applied Physics, University of Maryland, College Park, MD, ³Departments of Chemical and Biological Engineering; Genetics, Development and Cell, Biology, Iowa State University, Ames, IA, ⁴Moore's UCSD Cancer Center and Department of Reproductive Medicine, University of California, San Diego, La Jolla, CA

Focal Adhesions (FA) are mechanosensitive adhesion and signaling complexes that grow in size and change in composition in response to tensile forces in a process known as FA maturation. To better understand tension-mediated FA maturation, we sought to find FA proteins that are recruited to FA in cells in a myosin II-dependent manner, and to examine the mechanism for their myosin II-sensitive FA association. We find that the FA recruitment of the cytoskeletal adapter protein, vinculin and the focal adhesion tyrosine kinase, FAK is myosin II and ECM-stiffness dependent. We show that in cells, vinculin's association with paxillin, but not talin, is sensitive to myosin II activity, and that either paxillin or its homologue, Hic5 are important for vinculin recruitment to FA. Myosin II activity also promotes FAK/Src-mediated phosphorylation of paxillin on tyrosines 31 and 118. We show that phospho-mimic mutations of paxillin at tyrosines 31 and 118 can promote the recruitment of vinculin to adhesions, even in the presence of myosin II inhibitor. These results reveal an important role for paxillin, and its regulation by phosphorylation, in adhesion mechanosensing and maturation via myosin II-mediated FAK phosphorylation of paxillin, which generates binding sites for

vinculin to promote reinforcement of the cytoskeletal linkage and FA growth.

Fluorescent Probes of Membrane-bound Alpha-synuclein: Insights into the Role of Membranes in Aggregation. C. M. Pfeferkorn and J. C. Lee, Laboratory of Molecular Biophysics

Identifying pathways of alpha-synuclein (alpha-syn) misfolding are of great importance because the presence of aggregated alpha-syn in the brain is a hallmark of Parkinson's disease (PD). Due to the proximity of alpha-syn to synaptic organelles *in vivo*, the role of phospholipid membranes in modulating alpha-syn oligomer and aggregate formation is of particular interest. To probe how membranes affect α -syn conformation, we use circular dichroism spectroscopy as a secondary structural probe in conjunction with steady-state and time-resolved fluorescence techniques. Specifically, we employ single Trp-containing and dye labeled (1,5-IAEDANS, IANBD amide) alpha-syn variants to report on residue-specific environments and intermolecular contacts *via* changes in intrinsic fluorescence and measurements of Förster energy transfer between proteins modified with either donor or acceptor fluorophores. Using these methods, we aim to characterize the protein-protein and protein-membrane interactions that promote protein aggregation

Multi-Scale Modeling of Coarse Grained Protein Interactions: A CHARMMing Implementation. F. C. Pickard IV, B. T. Miller, H. L. Woodcock, H. F. Schaefer III, B. R. Brooks, Laboratory of Computational Biology

Off-lattice coarse grained (CG) models are an important class of computational tools that efficiently simulate both proteins and lipids. In the Klimov-Thirumalai (KT) implementation of the protein Gô model, each residue is represented by two interaction centers: one at the C α position and one at the side-chain center of mass position. This structure is biased towards its native state using 12-6 Leonard-Jones potentials to mimic the effects of hydrogen bonds and repulsive/attractive side chain interactions. This simplistic model allows for computational speedups of up to $\sim 10^3$ over their all-atom (AA) counterparts, while still retaining semi-quantitative agreement with respect to experimental kinetic and thermodynamic properties. The KT model was implemented in CHARMMing, a web-based graphical user interface for the CHARMM molecular simulation package, using Python. Through CHARMMing, users can specify many options of the KT model including: protein domain membership, non-bonded interaction potentials and various scaling factors. To validate the KT model, and its implementation, a mutant of the zipper domain of the yeast protein GCN4 was simulated at both AA and CG resolutions in a periodic cell with explicit solvent. Preliminary results are promising, as computed properties such as melting point and α helix orientation were consistent. This model will be extended to incorporate transferable parameters to inter-chain dynamics, while retaining the structure based approach for intra-chain dynamics.

Paxillin Phosphorylation Is a Local Regulator of Mechanotransduction Within Individual Focal Adhesions. S. V. Plotnikov¹, B. Sabass², A. Pasapera¹, U. S. Schwarz², C. M. Waterman¹, ¹Laboratory of Cell and Tissue Morphodynamics, ²University of Heidelberg, Heidelberg, Germany

The ability of eukaryotic cells to generate force and to sense the mechanical properties of the extracellular matrix (ECM) underlies many biological processes, such as cell migration, proliferation and differentiation. This is achieved in part by integrin-

mediated focal adhesions (FA), protein assemblies that couple contractile actomyosin bundles to the plasma membrane and transmit force generated by the cytoskeleton to the ECM. It has been demonstrated recently that protein composition and/or post-translational modification state can vary across an individual FA. However, whether this translates to variation in physiological properties and/or function for sub-FA domains is not known.

To probe traction distribution along single FA in migrating mouse embryonic fibroblasts, we used high-resolution traction force microscopy. We revealed that the region of maximum traction for the majority of FAs was skewed from the maximal paxillin-eGFP intensity toward their distal edges (cell periphery). The extent of skewness was significantly increased by plating cells on a soft substrate, but was independent from the functional state (growing, stationary, sliding or disassembling) of FA. We visualized dynamics of traction distribution along single FAs and revealed that mechanical coupling on integrin-actin interface is a stochastic process mediated by balance between cellular contraction and ECM stiffness. We tested that variation in force transmission across a FA is directly coupled to differences in biochemical composition across an individual FA. We found that perturbing the gradient of paxillin phosphorylation across FA by expressing Y^{31/118}E- or Y^{31/118}F-paxillin mutants or by inhibiting FAK homogenized adhesion population by eliminating fraction of strong adhesions. It also constrained maximum of traction to the center of FA. In agreement with stochastic clutch oscillation model, the asymmetry of traction distribution was rescued by decreasing either substrate stiffness or myosin II contractility. Since paxillin phosphorylation on tyrosine residues 31 and 118 mediates vinculin recruitment into FAs, we demonstrated that paxillin-vinculin interaction is essential for the asymmetric actin-integrin coupling. Thus, we proposed that enrichment of the distal tip of FA with phosphopaxillin is required for local force independent recruitment of vinculin into FAs. As soon as talin engages on actin-integrin interface, the pool of paxillin-bound vinculin reinforces actin-integrin linkage and stabilizes the clutch, which follows with actin retrograde flow. The physiological consequence of such asymmetric FA engagement to actin cytoskeleton was demonstrated to guide cell migration along rigidity gradient of ECM.

Inhibition of Lipid Synthesis Activates the DNA Damage Response and Triggers Premature Senescence in Human Fibroblasts. C. Quijano, L. Cao, J. Liu, M. Fergusson and T. Finkel, Translational Medicine Branch

Cellular senescence is a state of irreversible growth arrest with characteristic morphological and histological changes that include senescent associated β -galactosidase (SA- β -Gal) staining and the activation of the DNA damage response (DDR). Although initially considered the result of telomere shortening, senescence is now known to be triggered by a host of endogenous and exogenous stresses.

We sought to characterize the metabolic changes in human primary lung fibroblasts as they enter senescence. We noted that entry into senescence is accompanied by an approximate 60% decline in lipid synthesis, due in part to a decrease in fatty acid synthase (FAS) expression, and a corresponding increase in mitochondrial fatty acid oxidation, along with an increase in fatty acid dependent oxidant formation and p38 MAPK phosphorylation.

To determine if this metabolic shift, from lipid synthesis to oxidation, was associated with the onset of senescence, we directly inhibited fatty acid synthesis in fibroblasts with a pharmacological inhibitor of FAS (C75) or inhibited acetyl-CoA carboxy-

lase 1 (ACC1) expression using lentiviral delivery of ACC1 specific shRNA. Both treatments inhibited cell replication and markedly and rapidly increased the number of SA- β -Gal positive cells. Inhibition of lipid synthesis also resulted in nuclear 53BP1 foci and phosphorylation of H2AX, evidencing the activation of the DDR pathway. Since p38 was phosphorylated and active in the shACC1 cells, we incubated shACC1 cells with the antioxidant N-acetylcysteine or with SB203580, a specific pharmacological inhibitor of p38. Both treatments inhibited the increase in SA- β -Gal observed following the inhibition of lipid synthesis; suggesting that oxidant dependent p38 phosphorylation might be responsible for causing the premature senescence. Together, our results point to “metabolic stress”, in particular inhibition of lipid synthesis, as a new trigger for premature senescence and activation of the DDR in cells.

Investigating the Single Molecule Kinetics of Myosin 7a Using an Optical Trap. A. Roka¹, V.B. Siththanandan¹, Y. Takagi¹, Y. Yang¹, D.K.T. Hong², J.R. Sellers¹, ¹Laboratory of Molecular Physiology, ²Summit Computers, Washington DC

Myosins are a family of motor proteins ubiquitous in eukaryotic cells. Myosin 7a is an unconventional myosin that plays an important role in maintaining stereocilia rigidity. *Drosophila* myosin7a (Dm7a), a plus-end directed motor, has 5 calmodulin binding (IQ) domains which act as a lever-arm for the molecule, followed by a predicted single alpha-helix (SAH) domain, and a SH3 domain which separates two MyTH4-FERM domains. *Drosophila* myosin 7a mutants are generally lethal at larval stage, but escapers exhibit disorganized bristle structure and impaired auditory function. Previous work in our lab has shown full length Dm7a is auto-regulated through interaction of the tail end domain (C-terminal end) and the motor head (N-terminal end). Here we present single molecule kinetics and mechanics for Dm7aTD1, a truncated Dm7a construct with the 414 amino acids following the SH3 domain removed from the C-terminal. The Dm7aTD1 construct removes auto-inhibition and hence can be used to study the myosin's kinetics. Single molecule data were obtained using the “three-bead” assay using the dual-beam optical trap apparatus. The data from the assay were used to determine the actin detachment rate and step-size of the Dm7aTD1 construct. Our study suggests there is no significant change in the step-size of Dm7aTD1, calculated to be ~13 nm at both 10 μ M and 1 μ M ATP. The actin detachment rate is slow at low ATP concentrations and was calculated to be 1.2 s⁻¹ and 1.1 s⁻¹ at 1 and 10 μ M ATP, respectively. The single molecule kinetics data suggest that during its enzymatic cycle the myosin spends the majority of its time attached to actin filaments.

Improvement of Reverse Cholesterol Transport by Lcat Injection in Mice. X. Rousset, B. Vaisman, B. Auerbach, B. Krause, R. Homan, J.Stonik, A. Remaley Pulmonary and Vascular Medicine Branch

LCAT deficiency is associated with low HDL and the presence of the abnormal lipoprotein Lpx, which causes renal disease. LCAT by the esterification of cholesterol promotes reverse cholesterol transport and may, therefore, be anti-atherogenic. We examined the effect of the infusion of LCAT in mouse models. Intravenous delivery of LCAT (2500 nmol/ml/hr) in apoA-I transgenic mice increased HDL-C by 2.2-fold at 24h. When LCAT was infused in LCAT-KO mice, the large particles (VLDL and Lpx) rapidly disappeared and were largely converted to LDL. HDL-C had increased from 6 mg/dL to 40 mg/dL, then gradually decreased

to baseline by 72h. To study a possible synergistic effect of apoA-I and LCAT, we injected LCAT in LCAT-KO mice crossed with apoA-I transgenic mice. Compared to LCAT-KO mice, these mice had a smaller HDL peak but also a prominent pre- β HDL peak. Upon LCAT injection, plasma TC increased from 80 to 261 mg/dl at 48h, most of which was associated with HDL. The half-life of LCAT was 6.7h compared to 1.25h in LCAT-KO mice. Daily injection of LCAT in these mice for 4 days reduced the cholesterol content of the aorta from 14.58 ug/mg to 9.97 ug/mg (P<0.05, N=6). In summary, LCAT rapidly restored the abnormal lipoprotein phenotype in LCAT-KO mice and raised HDL-C in apoA-I transgenic mice. LCAT administration reduced the cholesterol content of aortas from LCAT-KO/apoA-I transgenic mice. These results suggest the feasibility of the use of LCAT for treating LCAT deficiency and for possibly increasing HDL-C to prevent and treat atherosclerosis.

Molecular Cloning, Expression and Enzyme Kinetics of C.elegans Phosphodiesterase 3, a Homolog of the Mammalian PDE3 Family. A. Samidurai¹, C. Tao², F. Ahmad¹, H. Liu¹ and V. C. Manganiello¹, ¹Translational Medicine Branch, ²Oral Infection and Immunity Branch, NIDCR

Cyclic nucleotide phosphodiesterases (PDEs) regulate the intracellular concentrations of the second messengers, cAMP and cGMP, by controlling their hydrolysis. Mammalian PDE3 is known to play an important role in insulin signaling pathways and in platelets, cardiovascular tissues, adipocytes, and oocytes

We report here studies of expression and characterization of the *C.elegans* Phosphodiesterase3 (CEPDE3) gene, a homolog of the mammalian PDE3 family. The nematode PDE3 gene is present on chromosome II, spanning about 22.2 Kb, and encodes two different CEPDE3 isoforms. The CEPDE3 long form (LF) consists of 11 exons and codes for a 63.5 kDa protein; the short form (SF) has 8 exons and codes for a 54.2 kDa protein. Both CEPDE3 isoforms have the characteristic mammalian PDE phosphodiester domains, and also contain the HD metal binding motif, which is unique for the PDE superfamily. The phylogenetic tree construct shows that CEPDE3 is close to mammalian PDE3. The predicted sequences of CEPDE3LF and SF isoforms show an overall 97 % homology between each other, with identical catalytic domains. An unique PDE3 signature sequence is also present in the CEPD3LF & SF. PDE activity assays indicated that recombinant CEPDE3 long and short forms are markedly inhibited by cilostamide (a specific inhibitor of mammalian PDE3), but not by rolipram (a specific inhibitor of PDE4). The IC50 values for cilostamide and rolipram inhibition are similar for recombinant CEPDE3 long form and CEPDE3 short form compared with the recombinant mammalian PDE3. The MS/MS sequence of purified recombinant CEPDE3LF contained several predicted phosphorylation sites. **Keywords:** PDE, cAMP, cGMP, *C.elegans*, CEPDE3, Pfam, catalytic domain, cilostamide, rolipram, inhibitor.

Motion Tracking Using Optical Navigation During *in-vivo* Two-Photon Microscopy. J. Schroeder¹, R. Pursley², M. Bakalar², T. Pohida², P. Kellman¹, R. Balaban¹, ¹Laboratory of Cardiac Energetics, NHLBI, ² Signal Processing and Instrumentation Section, CIT

We adapted a commercial multi-photon microscope to do real-time motion tracking of *in-vivo* tissue. An upright microscope was equipped with an objective piezo stepper motor to rapidly adjust focal plane. Image acquisition was performed with a resonant scanning mirror at 24 frames per second while the piezo motor

changed the focal plane of each frame. The resulting image sequence would oscillate focal plane up and down across a 20+ micron depth at a period of up to 3 Hz. The image stream was reassembled in real-time into a 3-D volume, and a parallel processing algorithm compared each new volume to a previously acquired reference volume to calculate offsetting motion and make compensatory adjustment of stage position. Using this system we tracked tissue during various physiological perturbations within several microns, and were able to observe dynamic physiological processes. Physiological perturbations included metabolic substrates, drug delivery, and anoxia. Image sequences were analyzed using principal component analysis to find significant trends and map them to spatial locations. Motion tracking expands the number of tissues and experimental preparations in which in vivo microscopic observation is possible.

MAT1, An Acetyltransferase Conserved From Prokaryotes, Regulates Mammalian Mitochondrial Respiration. I. Scott, B. R. Webster, M. N. Sack, Translational Medicine Branch

Lysine acetylation, using acetyl-CoA-derived substrates, is emerging as an important post-translational modulator of protein function. The addition (by acetyltransferases) or removal (by deacetylases) of acetyl groups from lysine residues can result in the activation or deactivation of a protein, lead to a change in its cellular localization, or even mark it for degradation. Acetylation is found in ~20% of the mammalian mitochondrial proteome, and has recently been shown to be a key regulator of respiration and ammonia detoxification. While several mitochondrial lysine deacetylases (e.g. SIRT3) have previously been identified, enzymes that perform the reverse reaction have not. Here we identify Mitochondrial Acetyltransferase 1 (MAT1) as the first mitochondrial lysine acetyltransferase. MAT1 is structurally related to known mammalian acetyltransferases but, interestingly, contains a catalytic domain conserved from prokaryotes, the ancestors of mitochondria. Using acetyl-CoA as a substrate, MAT1 acetylates electron transport chain proteins NDUF9 (complex I) and ATP synthase subunit alpha (complex V), down-regulating mitochondrial respiration. Knockdown of MAT1 reverses the mitochondrial protein hyperacetylation and respiration defect caused by SIRT3 deficiency, indicating that it acts in direct opposition to this deacetylase. We speculate that MAT1 and SIRT3 act as dynamic regulators of mitochondrial protein acetylation and, hence, mitochondrial function.

Single-Molecule Study of Human Topoisomerase II. Y. Seol¹, A. C. Gentry², N. Osheroff², and K. C. Neuman¹,¹National Institutes of Health, Bethesda, ²Vanderbilt University, Nashville, TN

Topoisomerase II is an essential enzyme that maintains genomic integrity by simplifying global DNA topology through supercoil relaxation, decatenation of linked chromosomal DNA, and DNA unknotting. This is achieved by generating a transient double-stranded break in one DNA segment through which a second double-stranded segment is passed prior to religation of the break. Failure or prevention of DNA religation leads to double stranded DNA breaks and fragmentation of the genome. Thus, the DNA cleavage reaction of human topoisomerase II (hTopo II) is one of the most successful targets for anti-cancer drugs. In order to develop effective anti-cancer drugs, it is critical to understand the detailed mechanism of DNA supercoil relaxation driven by hTopo II. One of the two isoforms of human Topoisomerase II, hTopo II α , preferentially relaxes positive supercoils, a feature it shares with only one other type II topoisomerase; *E. coli* topoisomerase IV.

Here, we have investigated the mechanism of DNA supercoil relaxation by hTopo II α using single molecule magnetic tweezers experiments. We measured the DNA relaxation rate of positive and negative supercoils and found that positive supercoil relaxation was ~2 times faster than negative supercoil relaxation. This result indicates that the relaxation mechanism of hTopo II α is different from that of topoisomerase IV, which displays an almost absolute preference for positively supercoiled DNA in single-molecule experiments. Our data further suggest that there is a small, ~30%, difference in the processivity of hTopo II α relaxing positive versus negative supercoils. These data, together with twist dependent relaxation rate measurements, allow a detailed comparison with the mechanism of chiral discrimination by *E. coli* topoisomerase IV, and suggest possible mechanisms of chiral discrimination by type II topoisomerases.

Synthesis of ⁶⁸Ga-Radiolabeled Proteins and Peptides for Positron Emission Tomography. N. Shenoy, J. Capala, G. Kramer, G. L. Griffiths, Imaging Probe Development Center at NHLBI and NCI

Positron emission tomography (PET) is an important imaging modality for preclinical and clinical quantitative imaging in a range of diseases. Biomolecules and small molecules radiolabeled with PET emitting radionuclides are increasingly used as diagnostic tools in disease detection and therapy evaluations. ⁶⁸Ga decays by positron emission with a half-life of 68 minutes and can be obtained from a ⁶⁸Ge/⁶⁸Ga radionuclide generator. This availability makes the ⁶⁸Ga readily available to facilities that lack a cyclotron and several generators are being developed with intent for clinical application. We have set up and operated a modern titanium oxide-based ⁶⁸Ge/⁶⁸Ga generator for ready supply of ⁶⁸Ga radionuclide and further coupled it to a remote, automated module for radiolabeling of targeting moieties. This tandem system is under consideration for application for ultimate FDA submission by its manufacturer, Eckert & Ziegler. We will describe operation and quality performance of the system particularly with reference to the generation of two target-specific agents, an anti-EGFR Affibody[®] molecule and the somatostatin receptor targeting agent, Octreotide[®]. Affibody molecules are a class of 3-helix protein scaffolds derived from an IgG binding domain of staphylococcal protein A. Reaction parameters for the radiolabeling of bifunctional chelate DOTA-derivatized Affibody molecules and Octreotide with ⁶⁸Ga were optimized to obtain high radiolabeling efficiencies. Biodistribution and imaging studies of ⁶⁸Ga- DOTA-derivatized Affibody molecules in mice bearing HER2 receptor expressing tumor models are underway, and the generator system is available to other users within NIH.

Large Scale Profiling of Protein Degradation Rates and Translation Rates In Renal Collecting Duct Cells. D. H. Slentz, M. J. Yu, T. Pisitkun, J. D. Hoffert, M. A. Knepper. Epithelial Systems Biology Laboratory

In a given cell type, steady-state protein abundance is determined by a balance between rates of protein production and degradation. Long-term exposure of collecting duct principal cells to the antidiuretic hormone arginine-vasopressin (AVP) causes an increase in abundance of the water channel protein aquaporin-2, a process critical to regulation of water excretion. However, whether the abundances of other proteins undergo parallel changes is unknown. Furthermore, mechanisms of abundance changes have not been explored. To carry out global measurements of protein half-life ($t_{1/2}$) and relative translation rate ($R_{t(rel)}$) for large numbers

of expressed proteins in cultured renal mpkCCD cells, we have employed stable isotope labeling (SILAC) coupled with protein mass spectrometry (LC-MS/MS) in the presence and absence of vasopressin. A mathematical model based on mass balance equations, assuming first order kinetics of degradation, provided the means of calculating $t_{1/2}$ and $R_{t(\text{rel})}$ for each identified protein. Initial experiments ($n=3$) have provided $t_{1/2}$ measurements for 1,068 proteins, a small fraction of which were found to change in response to vasopressin. The half-life of aquaporin-2 was unchanged. Translation rate measurements have been completed in one experiment. Among all proteins quantified, aquaporin-2 appeared to show a large increase in translation rate, which correlates with the previous finding of a large increase in aquaporin-2 mRNA levels in response to vasopressin. With analysis of results and correlation with transcript levels, we expect to learn what fraction of proteins undergoes post-transcriptional regulation of protein abundance and an assessment of the relative importance of translation regulation versus regulation of protein degradation.

Maximizing Signals from in vivo Multiphoton Microscopy: Non-contact Total Emission Detection (epi-TED). A. V. Smirnov¹, C. A. Combs², David Chess³, M. Luger-Hamer³, D. B. McGavern⁴, S. S. Kang⁴, J. R. Knutson¹, and R. S. Balaban³, ¹Laboratory of Molecular Biophysics, ²Light Microscopy Facility, ³Laboratory of Cardiac Energetics, ⁴NINDS Viral Immunology and Intravital Imaging Unit

The generation of a privileged focal volume for two-photon excitation of fluorophores within living tissue is an imaging event. The scanned collection of fluorescence emission is incoherent; i.e., no real image needs to be formed on a detector plane. Thus, new schemes that efficiently funnel all attainable photons to detector(s) become tenable. We previously showed (JOM v.228, p.330-7, 2007) that parabolic mirrors and condensers could be combined to collect the totality of solid angle around the spot for tissue blocks, leading to ~8-fold signal gain.

We now show a new version of this Total Emission Detection instrument modified to make non-contact images inside tissue in vivo. The device is mounted on a periscope (LSM Tech) to facilitate approach to live animals. We find that blood vessels tagged with DsRed-actin ~100 μm deep within an exposed mouse brain provide over 2.5X more light in the TED-II device (compared to light collected by the 20X water 0.95NA dipping objective alone).

Thus, scanning with the same SNR could occur at more than twice the normal rate. Alternatively, one could reduce laser power 60% to reduce photodamage. We have also designed a smaller version to directly replace an objective.

Novel Mutations in *NT5E* Identified in Adults with Arterial Calcifications. C. St. Hilaire, S. G. Ziegler, T. Markello, A. Bruscs, C. Groden, F. Gill, H. Carlson-Donohoe, R. J. Lederman, M.Y. Chen, D. Yang, M.P. Siegenthaler, C. Arduino, C. Mancini, B. Freudenthal, H.C. Stanescu, A.A. Zdebik, R. Krishna Chagant, R. Nussbaum, R. Kleta, W.A. Gahl, M. Boehm, Translational Medicine Branch

We identified nine otherwise healthy individuals in three families with *de novo* arterial calcifications in their lower extremity arteries and joint capsules. Genetic analysis identified three novel mutations in the *NT5E* gene of affected individuals of the three families. *NT5E* encodes for CD73, an ecto-5-prime-nucleotidase that converts extracellular AMP to adenosine. All identified mutations result in nonfunctional CD73. Adenosine acts as a signaling molecule by binding to one of the four adenosine receptors ex-

pressed on a wide range of cells; these patients are assumed to have reduced adenosine signaling, as CD73 is the major enzyme that metabolizes AMP to adenosine in the extracellular milieu. The role of adenosine signaling in pathological vascular calcification is currently unknown. Cultured fibroblasts of affected individuals exhibited markedly reduced expression of *NT5E* mRNA, CD73 protein, and enzyme activity; cultured fibroblasts displayed increased alkaline phosphatase levels and accumulated calcium phosphate crystals. Genetic rescue experiments normalized CD73 activity in patient cells, and adenosine treatment reduced alkaline phosphatase levels. Pathological vascular calcification is normally presented as a secondary complication to diseases such as atherosclerosis, diabetes mellitus type II, and chronic kidney disease. Recent studies suggest calcification in the vasculature mimics bone formation, however it is unclear as to the precise mechanisms involved; we have identified CD73 as a potential regulator of this pathology. Loss of CD73 function due to biallelic *NT5E* mutations represents a new autosomal recessive disorder of vascular calcification, revealing the critical role of adenosine in inhibiting ectopic calcification within specific vessels and joints.

Pink1 Preserves Cardiac Function in Response to Pressure-Overload and Aging Induced Stress Through Regulating Mitochondrial Dynamics. M. V. Stevens, K. Y. Kim, D. Springer, S. Andersen, A. Noguchi, S. Esfahani, M. Daniels, H. San, M. N. Sack, Translational Medicine Branch

The phosphatase and tensin homologue deleted on chromosome 10 (PTEN) induced putative kinase-1 (Pink1) is associated with resilience to cardiac redox stress, although the pathways underpinning this phenotype are unknown. As neuronal depletion of Pink1 results in increased mitochondrial fragmentation, we postulated that Pink1 may function to modulate cardiac mitochondrial dynamics. We tested this by comparing wildtype and Pink1^{-/-} mice during an acute inotropic challenge, in response to chronic transverse aortic constriction (TAC)-induced pressure-overload, and aging. Basal contractile function was similar between the mouse strains and both strains showed robust augmentation in ejection fraction and heart rate in response to a dobutamine challenge as measured by cardiac MRI. In response to 8 and 12 weeks of TAC, ejection fraction significantly decreased, with an increase in left ventricular cavity size in knockout versus wildtype mice. This cardiac decompensation is associated with increased myocardial fibrosis and evidence of an increased number of smaller mitochondria in the myocardium compared to wildtype control mice. Interestingly, Pink1^{-/-} mice at 6 months of age exhibited decreased ejection fraction and increased ventricular dilatation compared to wild-type controls. Steady-state protein levels of modulators of mitochondrial fission including DRP1 and Fis1 are significantly elevated in pressure-overloaded knockout mouse myocardium compared to controls. In parallel, phenylephrine-induced rat neonatal cardiomyocyte hypertrophy exhibit decreased Pink1 and increased Drp1 expression in contrast to vehicle treated controls. The signaling pathways controlling this program are being explored. We conclude that Pink1 functions to maintain mitochondrial homeostasis and fusion in response to chronic pressure-overload and aging in the heart. In contrast, Pink1 is not required to adapt to an acute energetic demand as evident by equal responsiveness to inotropic challenge. Together these findings suggest that the control of mitochondrial dynamics are not necessary in cardiac adaptation to acute energy demand in young mice, but is required to enable adaptive remodeling following chronic pressure-overload and in response to aging.

Gold Nanoparticles as Potential Cancer Imaging and Therapeutic Agents. A. Sulima, B. Xu, N. Shenoy, G. L. Griffiths, J. Capala, G. Kramer-Marek, The Imaging Probe Development Center at NHLBI in collaboration with NCI

Nanoparticles, engineered as platforms for effective and targeted therapy, drug delivery and imaging labels have been actively pursued for biomedical applications in recent years. Gold nanoparticles (AuNPs) are among the most studied and at the Imaging Probe Development Center (IPDC) we are developing gold nanoparticle-based agents for cancer imaging and therapy. Preparation and characterization of multifunctional poly(ethylene glycol)-grafted gold nanospheres will be reported in detail along with a discussion of their possible use in both molecular imaging and radiotherapy. Surface modification of AuNPs with thioctic acid functionalized poly(ethylene glycol), (TA-PEG5,000), effectively reduces their nonspecific interactions with proteins and suppresses particle uptake by the reticuloendothelial system (RES). In addition, bifunctional PEG allows for conjugation of probe molecules (targeting ligands, drugs or imaging labels) to nanoparticles. For the targeted delivery of the gold nanoparticles, Affibody Molecules® will be conjugated to AuNPs through a bifunctional PEG linker. The Affibody molecules employed in this study selectively bind the human epidermal growth factor receptor-2 (HER-2), which is overexpressed in certain breast and lung tumors. For fluorescence imaging purposes AuNPs were further functionalized with a NIR dye, Bodipy FL. Biological evaluation of targeted versus non-targeted gold nanoparticles as well as biodistribution and imaging studies are currently ongoing at the National Cancer Institute.

Ca²⁺ Sensing Receptor (CaSR): a Mediator of Ischemic Preconditioning in the Hearts? J. Sun, E. Murphy, Translational Medicine Branch

As a G protein-coupled receptor (GPCR), the extracellular Ca²⁺-sensing receptor (CaSR) responds to changes not only in extracellular Ca²⁺ but also to many other ligands, elicits complex intracellular signaling transduction. CaSR has been found to be expressed in the hearts, however, its physiological function is not clear. In this study, we investigated whether CaSR plays a cardioprotective role in ischemic preconditioning (IPC). Hearts from C57BL/6J mice (male, ~15 weeks) were perfused in the Langendorff mode and subjected to the following conditions: (1) control perfusion; (2) 30 min of perfusion with a highly specific CaSR antagonist, NPS2143; (3) IPC (four cycles of 5 min of global ischemia and 5 min of reperfusion); or (4) 10 min of perfusion with NPS2143 prior and during IPC. Then hearts were subjected to 20 min of no-flow global ischemia and 120 min of reperfusion. Compared with control, IPC significantly improved postischemic left ventricular functional recovery and reduced infarct size. Although NPS2143 perfusion alone did not change the hemodynamic function and did not change the level of post-ischemic injury, NPS2143 treatment totally abolished cardioprotection of IPC. Through Western blot analysis, it was demonstrated that IPC significantly increased the levels of phosphorylation of ERK1/2, Akt, and GSK-3 β , suggesting IPC lead to the activation of these signaling pathways. Taken together, the inhibition of CaSR by NPS2143 treatment abolished IPC-mediated cardioprotection, suggesting the activation of CaSR during IPC is cardioprotective. Thus, CaSR in the cardiomyocytes could sense the ischemic stress signal during IPC and mediate the downstream cardioprotective signaling.

Mitochondrial respiration Protects Against Oxygen-associated DNA Damage. H. J. Sung, W. Ma, P. Wang, J. Hynes¹, T. C. O'Riordan¹, C. A. Combs², P. J. McCoy³, F. Bunz⁴, J. Kang and P. M. Hwang, Translational Medicine Branch, ¹ Luxcel Biosciences Ltd., Ireland, ² Light Microscopy Core Facility, ³ Flow Cytometry Core Facility, ⁴ Radiation Oncology Department, Johns Hopkins University School of Medicine

Oxygen that sustains aerobic life through oxidative phosphorylation also serves as the essential substrate for the formation of reactive oxygen species (ROS), implicated in aging and tumorigenesis. Although the mitochondria is best known for its bioenergetic function, the symbiotic theory originally proposed that it provided protection against the toxicity of increasing oxygen in the primordial atmosphere. We sought to genetically test the oxygen toxicity hypothesis by disrupting a gene essential for respiration, Synthesis of Cytochrome c Oxidase 2 (SCO2). Human cells deficient in SCO2 (SCO2^{-/-}) are oxidative phosphorylation-defective and glycolysis-dependent, exhibit increased viability under conditions of hypoxia and feature an inverted growth response to oxygen compared to wild-type cells (SCO2^{+/+}). Notably, these respiration-defective cells have increased intracellular oxygen and NADH levels which result in increased reactive oxygen species (ROS) and oxidative DNA damage. Thus, by creating an isogenic human cell line that is energetically independent from oxygen for growth, we have revealed the underlying genotoxicity of ambient oxygen. Our study highlights the importance of mitochondrial respiration both for deriving bioenergetic benefits and for maintaining genomic stability in an oxygen-rich environment, and may provide basic insights for cancer prevention strategies.

Glypicans Coordinately Regulate Zebrafish Dorsal-ventral Development. C. Tharp, A. Srinivasan, K. L. Kramer; Developmental Glycobiology Section

Heparan sulfate proteoglycans are essential molecules that mediate multiple cell-cell signaling pathways during embryonic development. Signaling molecules, receptors, or agonists/antagonists can directly interact with core proteins or their attached heparan sulfate chains. To better understand how glypicans (GPCs)- a membrane anchored subfamily of core proteins-function in zebrafish development, we used antisense morpholinos to individually and specifically knock down the expression of all ten GPC genes in zebrafish. Knockdown of GPC2, GPC6a, or GPC6b resulted in strong dorsal-ventral phenotypes between gastrulation and 24hpf. To better understand the molecular pathways in which GPCs are involved, we used a candidate gene approach to study changes in transcription of BMP, Wnt and FGF target genes because these cell-cell signaling pathways are known to regulate dorsal-ventral patterning. Using QPCR, we identified seven genes that are significantly up-regulated in at least one of the three GPC knockdowns. The gene expression patterns were different between each GPC knockdown, with GPC2 having a distinct affect on BMP2b expression while GPC6a and 6b differentially increased expression of *Ved/Vox/Vent*. These results were confirmed by *in situ* hybridization, demonstrating that BMP2b expression is significantly expanded to the dorsal side in GPC2 morphant embryos. Our preliminary results suggest that each of the three GPCs studied regulates distinct steps in dorsal-ventral patterning, an observation that would add a new dimension to our understanding of how proteoglycans might work together to mediate early development.

Vinculin Stabilizes Nascent Adhesions and Establishes a Lamellipodium-lamella Border in Migrating Cells. I. Thieversen, S. Berlemont, A. Zemljic-Harpf, R. S. Ross, C. M. Waterman,

Laboratory for Cell and Tissue Morphodynamics

The actin cytoskeleton at the leading edge of migrating cells consists of two actin networks, the lamellipodium (LP), characterized by fast polymerization-driven retrograde actin flow and the lamella (LM) with slow myosin II (myoII) mediated actin flow. The engagement of LP actin to the ECM via nascent integrin-mediated focal adhesions (FA) establishes the flow velocity gradient between LP and LM. Nascent adhesions then elongate and mature via myoII LM actin flow. How integrins are connected to the retrograde actin flow is not known. Using primary murine embryonic fibroblasts (MEF) with cre-mediated excision of the vinculin gene (Vcl), we sought to test the hypothesis that vinculin mediates the coupling of actin retrograde flow to the ECM in nascent FA. To determine if LP/LM organization was affected by loss of Vcl protein, we analyzed distributions of phospho-myosin light chain (pMLC), cortactin, and paxillin. This revealed a shift in pMLC distribution towards the cell edge, reduced LP paxillin intensity, and broadening of the cortactin band at the leading edge, suggesting a loss of delineation between the LP and LM. To test this, we performed total internal reflection (TIRF) and spinning disc confocal (SDC) microscopy of MEF containing fluorescent paxillin and actin. TIRF microscopy of EGFP-paxillin and mApple-actin showed a reduction in the rate of formation of short-lived, diffraction limited FA in the LP of Vcl^{-/-} MEF, indicating an impaired stabilization of nascent LP FA. Quantitative fluorescent speckle SDC microscopy of Xrhodamine-actin revealed increased retrograde f-actin flow velocities in both LP and the LM region of Vcl^{-/-} MEF. We suggest that vinculin stabilizes nascent FA by coupling to lamellipodial actin flow, thus establishing the flow velocity gradient between LP and LM and promoting the maturation of nascent adhesions. This implicates vinculin as an essential component linking the dynamic actin cytoskeleton to the ECM during cell migration.

Requirement of Vascular Niche for Adult Neurogenesis in the Subventricular Zone of Lateral Ventricle Wall of Brain. Y. Uchida, Y. Mukoyama, Laboratory of Stem Cell and Neuro-Vascular Biology

Neurons continue to be generated in adult brain as well as in embryonic nervous system. In adult brain, neurogenesis occurs in only two regions: the subventricular zone (SVZ) of lateral ventricle wall and the subgranular zone (SGZ) of hippocampus dentate gyrus. Neural stem cells (NSCs), self-renewing progenitor cells with the capacity to generate multiple differentiated neurons and glia, are presumed to exist in specialized microenvironments, or niches, that regulate their function. Emerging evidences has suggested that blood vessels may play key regulatory roles as NSC niches in these neurogenic regions. Indeed, we have developed a whole-mount staining system of the dissected SVZ and SGZ tissues to visualize a close proximity of NSCs and capillary-like blood vessels. To further examine whether blood vessels are required for the maintenance of adult neurogenesis in these neurogenic regions, we have taken advantage of the Cre-mediated inducible cell ablation system based on transgenic expression of diphtheria toxin receptor (DTR) and local administration of diphtheria toxin (DT). In Tie2-Cre, endothelial-specific Cre deleter line, with the Cre-mediated DTR transgenic mice, we observed apoptotic endothelial cells in the SVZ vasculature after stereotaxic DT injection. We are now extensively working to confirm that the endothelial cell ablation impairs adult neurogenesis in the SVZ region.

Characterization of the Interaction Between BetaPix and PDZ Domain of Sorting Nexin 27 in Kidney Epithelial Lysate J. L. Valdes, M. P. Playford, S. L. Milgram, Laboratory of Epithelial Cell Biology

Sorting Nexins are a family of proteins characterized by the presence of phosphoinositide binding Phox homology (PX) domain. Sorting Nexin 27 (SNX27) is a 62-kda protein, localized to early and recycling endosomes, involved in endosomal trafficking. It is composed of PDZ, PX and FERM/RA domains. To identify novel SNX27 binding partners, we performed a proteomic screen in mouse principal kidney cortical collecting duct cells (MPKCCD). To facilitate this screen, we created a glutathione s-transferase (GST) fused construct of the SNX27 PDZ domain and performed GST pull downs in MPKCCD lysates. We found that β -pix, which functions as a guanine exchange factor for the Rho family of small GTPases, interacted with SNX27. The association of β -pix and SNX27 has been confirmed by GST pull downs and co-immunoprecipitation assays. We have observed that the interaction of SNX27 and β -pix is abolished in cells expressing PDZ domain mutants. These data suggests that the PDZ domain is required for β -pix to associate with SNX27. We hypothesize that the PDZ domain of SNX27 binds to the carboxy-terminus of β -pix, which contains the type-1 PDZ binding motif: ETNL. To further test this hypothesis, we are investigating the localization of β -pix and SNX27 in polarized epithelial cells.

Production and Preliminary Testing of Ultra-Stable Gadolinium-Benzyl-DOTA-Cholera Toxin B Conjugates as MRI Brain Circuitry Tracking Agents. O. Vasalatiy, S. Cheal, C. W. H. Wu, R. B. Tootell, A. P. Koretsky, L. Ungerleider, G. L. Griffiths, The Imaging Probe Development Center at NHLBI in collaboration with NINDS & NIMH

Cholera toxin subunit B (CTB) is a useful agent for MRI imaging of brain circuitry by virtue of its transport properties. CTB labeled with gadolinium (Gd) may allow us to non-invasively image neuronal connections over extended time periods. Moreover it may have advantages over low molecular weight Gd complexes of DOTA-biotin, or over the toxic manganese chloride. Gd in chelated form, covalently attached to CTB by a bifunctional version of the macrocyclic chelating agent 1,4,7,10-tetraazacyclododecane-N,N',N'',N'''-tetraacetic acid (DOTA), should represent an optimum CTB contrast reagent useful for multi-day tracking, by virtue of its extreme *in vivo* stability. The Gd complex of the 4-isothiocyanatobenzyl-DOTA pre-activated bifunctional chelate was prepared and conjugated to CTB at Gd-chelate:CTB ratios ranging from 1.4 to 4.0, as we sought to balance maximum Gd content for ideal signal detection with maximum retention of CTB biological transport activity. Conjugates were comprehensively characterized for protein concentration and Gd content by MADLI-TOF, HPLC and ICP-MS, and the synthesis of the reagent and its physical and *in vitro* properties will be described.

MicroRNAs are Transported and Delivered to Recipient Cells by High Density Lipoproteins. K. C. Vickers, B. T. Palmisano, A. T. Remaley, Lipoprotein Metabolism Section

In the presence of cations, RNA can bind to phosphatidylcholine, and synthetic HDL-like liposomes incorporated with short interfering RNAs (siRNA) have been utilized as delivery vehicles for both *in vitro* and *in vivo* transfection studies. Here we present evidence demonstrating that human and mouse high-density lipoproteins (HDL) transports and delivers endogenous miRNAs. Highly purified fractions of HDL were prepared from human and

mouse plasma and purified total RNA was short in length (<30nt) and devoid of long mRNA. The isolated human HDL-miRNA profile was significantly different from the miRNA profile observed in exosomes of matched individuals ($R=0.102$, $p<0.05$), and the HDL-miRNA profile from plasma of normal subjects ($n=6$) was significantly different ($R=0.064$, $p<0.05$) than the HDL-miRNA profile observed in familiar hypercholesterolemia (FH) subjects ($n=6$). miRNAs were demonstrated to associate with native HDL particles as evidenced by gold-enhanced electron microscopy, fluorescent and radiolabeled gel filtration shift assays, and qPCR. Furthermore, synthetic naked HDL injected into mice retrieved distinct miRNA profiles from both normal and atherogenic (ApoE^{-/-}, high fat diet) models after 6hrs that correlated to normal and diseased human profiles. Finally, normal and diseased HDL delivered distinct miRNAs signatures to recipient cells with functional consequences resulting in significant differential gene expression changes. HDL-mediated delivery of endogenous and exogenous miRNAs is scavenger receptor BI-dependent. Collectively these observations suggest that HDL may participate in a novel mechanism of cellular communication, involving post-transcriptional gene regulation by miRNAs.

The Class V Myosin MYO5A Transports the Endoplasmic Reticulum into the Dendritic Spines of Purkinje Neurons. W. Wagner, J. A. Hammer III, Laboratory of Cell Biology

The presence of endoplasmic reticulum (ER) within the dendritic spines of Purkinje neurons (PNs) is required for cerebellar long-term depression, a form of synaptic plasticity that underlies motor learning. Previously, it was found that the ER is absent from the spines of PNs from mice with mutations in the class V myosin MYO5A. Here, we analyzed how the ER is targeted to PN spines and the role that MYO5A plays in this process. To this end, we developed an efficient method to express cDNAs in a PN-specific fashion in heterogenous, dissociated cerebellar cultures. Live imaging of wild type and *myo5a*-null PNs expressing both an ER- and a cell volume-marker shows that the myosin is required for the movement of ER into spines. Rescue experiments with *myo5a*-null cerebellar cultures indicate that MYO5A functions within the PN to mediate ER targeting, and that it must be able to hydrolyze ATP to rescue. Moreover, MYO5A concentrates at the ER's leading tip as the organelle moves into the spine, consistent with the myosin transporting ER. Finally, attenuation of the myosin's ability to move along actin filaments reduces the maximum velocity of ER movement into PN spines, providing direct evidence that MYO5A drives this ER motility. Therefore, while it has been proposed that type V myosins localize organelles by tethering them to the actin-rich cell periphery, we demonstrate here that MYO5A is a point-to-point organelle transporter that moves ER as cargo into PN spines. These results uncover the mechanism that mediates ER localization to PN spines, a prerequisite for synaptic plasticity.

Extracellular Matrix Remodeling and Hif-1 Signaling: New Insights From A Patient With Prolidase Deficiency. A. D. Walts, C. St. Hilaire, D. Kastner, I. Aksentijevich, M. Boehm, Translational Medicine Branch

Extracellular matrix remodeling plays a major role in many diseases as well as wound healing. A young NIH patient with an unknown disease presented with multiple lower limb ulcers leading to the amputation of several toes. Genetic analysis determined that this patient suffers from prolidase deficiency. Prolidase deficiency is commonly characterized by severe lower limb ulcers,

mental retardation, and facial abnormalities, and can be diagnosed by the presence of glycine-proline dipeptides in urine. It is an extremely rare autosomal recessive disorder that shares common clinical traits with lupus, scleroderma, and hyper-IgE syndrome. Prolidase is required for proper extracellular matrix remodeling and recycling of proline for collagen biosynthesis, however the molecular mechanisms of the disease are unknown. Matrix metalloproteases (MMPs) break down collagen into dipeptides. Prolidase is responsible for the further breakdown of X-Pro dipeptides into single amino acid units. Fibroblasts isolated from our patient have no TNF α -dependent expression of multiple MMPs, vEGF receptor 2, and endothelin 1. We show that prolidase deficient cells can be supplemented with Hydroxy-Proline (HyPro), a product of the prolidase metabolism of Glycine-HyPro dipeptides, resulting in the transcriptional restoration of the aforementioned genes. It has previously been shown that HyPro can stabilize hypoxia-induced factor (Hif-1). Consistent with a lack of HyPro recycling, our patient shows diminished activation of Hif-1. These results suggest that prolidase deficiency is an extracellular matrix remodeling disease in which the failure to activate Hif-1 is responsible for the decreased remodeling. In addition, these results begin to characterize the molecular mechanisms underlying prolidase deficiency and suggest HyPro as a possible treatment for the chronic ulcers associated with the disease.

Polo-Like Kinases Mediate Cell Survival In Mitochondrial Dysfunction. P. Wang, T. Matsumoto, W. Ma, H. J. Sung, S. Matoba, and P. M. Hwang, Translational Medicine Branch

Cancer cells are often defective in mitochondrial respiration, thus identification of pathways that promote cell survival under this metabolic state may have therapeutic implications. Here, we report that the targeted ablation of mitochondrial respiration markedly increases expression of Polo-like kinase 2 (PLK2), which is essential for survival of these non-respiring cells. Furthermore, we identified PLK2 as a kinase that phosphorylates Ser-137 of PLK1, which is sufficient to mediate this survival signal. *In vivo*, depletion of PLK2 in an isogenic human cancer cell line with a modest defect in mitochondrial respiration eliminates xenograft formation, indicating that PLK2 activity is required for growth of tumor cells with compromised respiration. Our findings delineate a mitochondrial retrograde signaling pathway that is critical for determining cancer cell outcome.

Stimulation of Nitric Oxide Synthase by Plasma From Patients With Sickle Cell Disease Is Associated With Endothelial Function. X. Wang, L. Freeman, B. Vaisman, A. Remaley and G. J. Kato, Pulmonary and Vascular Medicine Branch

Nitric oxide (NO) plays a critical role in maintaining basal vascular tone and regulating blood flow. Many factors, including shear stress and endogenous ligands such as vascular endothelial growth factor (VEGF) and insulin-like growth factor-1 (IGF1), stimulate the activity of endothelial nitric oxide synthase (eNOS). High density lipoprotein (HDL) is an important atheroprotective component of plasma. It has been previously shown that HDL stimulates eNOS activity in cultured cells. We have developed an assay in cultured endothelial cells that measures stimulation of NOS activity by unfractionated human plasma. We have found that as low as 4% plasma stimulated NO production quite well, 3 to 4-fold over background. We find that increased NO production is correlated in part with the presence of HDL proteins apolipoprotein A-I (apoA-I) and paroxonase-1 (PON1) by plasma fractionation,

indicating that a major component of NOS stimulating activity may be mediated by HDL. However, plasma from HDL-deficient apoA-I knockout mice still stimulates NOS activity significantly, only partially reduced compared to wild type mice and apoA-I transgenic mice, indicating the presence of additional NOS stimulating factors in mouse plasma besides apoA-I HDL. We find that the plasma NOS-stimulating activity correlates significantly with endothelial-dependent vasodilation in blood flow physiology assays in patients with sickle cell disease. These results suggest that the plasma NOS stimulation assay may be a useful surrogate assay for endothelial function, applicable to frozen archived plasma samples.

How Do the Co-repressor Hdacs Act in the Human Genome. Z. Wang¹, C. Zang², K. Cui¹, W. Peng², and K. Zhao¹, ¹Laboratory of Molecular Immunology, ² Physics Dept., George Washington University

Histone acetyltransferase (HAT) and deacetylase (HDAC) are two groups of enzymes with antagonizing function to control acetylation, which is critical for any organisms to precisely modulate gene expression, either activation or repression. Abnormality of this process may result in human diseases including cancers. As acetylation is a histone mark for active transcription, HATs, co-activators of transcription, have been associated with active and HDACs, co-repressors of transcription, with inactive genes, respectively. This so-called “On/off” model has been used to interpret data in numerous investigations.

Using ChIP-Seq, we mapped the genome-wide distribution of HATs and HDACs in the human genome and found that both bind to active genes with acetylated histones. The majority of HDACs, including Class I, II, and III HDACs, function to reset the chromatin by removing acetylation at active genes. Inactive genes that are primed by H3K4 methylation marks are subject to a dynamic cycle of acetylation and deacetylation by transient binding of HATs/HDACs, which prevents the Pol II binding but poises them for future induction. In contrast to the traditional “On/off” model, we found that constitutively silent genes without any H3K4 methylation signals show no evidence of being bound by co-repressor HDACs. We also found that the recruitment of HDAC6 and TIP60 to gene body is mediated through the interaction with elongating Pol II. In summary, our discoveries provide new insights into the function of HATs and HDACs. The emerging new concept/functional role of co-repressor HDACs provides a new perspective to understand the mechanisms of HDAC inhibitors in treating human diseases.

Mitochondrial Acetyltransferase I (Mat1) May Function as a ‘Nutrient Sensor’ Regulating Autophagy. B. R. Webster, I. Scott, M. V. Stevens, M. N. Sack, Translational Medicine Branch

Protein acetylation is a major post-translational modification on par with protein phosphorylation by the kinome. The majority of mitochondrial enzymes are acetylated included enzymes involved in fatty acid oxidation, the TCA and urea cycles, and the electron transfer chain. Given the central role of the mitochondria in metabolism, mitochondrial protein acetylation and subsequent effects on global metabolism have garnered much interest. The only known regulators of mitochondrial acetylation are the sirtuin NAD⁺ dependent deacetylases. We believe that we have found the first mitochondrial acetyltransferase (MAT1) to counter deacetylation. MAT1 localizes to the mitochondria and uses acetyl-CoA to acetylate mitochondrial proteins. Interestingly, during starvation the nutrient sensitive Sirtuin deacetylases mediate autophagy to facilitate the digestion of cellular components

thereby recycling nutrients for cellular preservation. We hypothesized that MAT1 similarly functions as a nutrient sensor in a ‘yin-yang’ fashion to the mitochondrial sirtuins. The genetic depletion of MAT1 should mimic sirtuin activation with the induction of mitochondrial autophagy. siRNA knockdown of MAT1 in HepG2 cells decreases mitochondrial protein acetylation and increases formation of vesicles containing glycogen, a potential sign of augmented autophagy. Isolated mitochondria from siRNA treated cells showed increased levels of the autophagy markers LC3-II, p62 and ubiquitin. Finally EM images support increased levels of autophagosomes in siRNA treated cells. Taken together, we hypothesize that MAT1 knockdown yields unopposed deacetylase activity initiating survival programs including glycogen sequestration and mitophagy. Future studies will better assess the ‘yin-yang’ paradigm of MAT1 and deacetylases and their influence on autophagy in-vitro as well as in-vivo.

No Evidence for Clonal Selection Due to Lentiviral Integration Sites in Human Induced Pluripotent Stem Cells. T. Winkler, A. Cantilena, J. Y. Métais, X. X. A. D. Nguyen, B. Borate, J. E. Antosiewicz-Bourget, T. G. Wolfsberg, J. A. Thomson, C. E. Dunbar, Hematology Branch

Derivation of induced pluripotent stem (iPS) cells requires the expression of defined transcription factors (among Oct3/4, Sox2, Klf4, c-Myc, Nanog and Lin28) in the targeted cells. Lentiviral or standard retroviral gene transfer remains the most robust and commonly used approach. Low reprogramming frequency overall, and the higher efficiency of derivation utilizing integrating vectors compared to more recent non-viral approaches suggests that gene activation or disruption via proviral integration sites (IS) may play a role in obtaining the pluripotent phenotype. We provide for the first time an extensive analysis of the lentiviral integration profile in human iPS cells. We identified a total of 78 independent integration sites (IS) in 8 recently established iPS cell lines derived from either human fetal fibroblasts or newborn foreskin fibroblasts after lentiviral gene transfer of Oct4, Sox2, Nanog, and Lin28. The number of IS ranged from 5 to 15 IS per individual iPS clone and 75 IS could be assigned to a unique chromosomal location. The different iPS clones had no IS in common. Expression analysis as well as extensive bioinformatic analysis did not reveal functional concordance of the lentiviral targeted genes between the different clones. Interestingly, in 6 of the 8 iPS clones some of the IS were found in pairs, integrated into the same chromosomal location within six base pairs of each other or in very close proximity. Our study supports recent reports that efficient reprogramming of human somatic cells is not dependent on insertional activation or deactivation of specific genes or gene classes.

Identifying Components of the Mitochondrial Permeability Transition Pore and Its Physiological Role Through Cyclophilin D Interactions. R. Wong, G. Wang, M. Gucek, J. D. Molkenin, C. Steenbergen, E. Murphy, Translational Medicine Branch

The mitochondrial permeability transition (MPT) pore is a conductance channel that mediates cell death and is proposed to be a Ca²⁺-release valve, but its identity is still unknown. MPT inhibitors, such as cyclosporin, reduce ischemia-reperfusion injury. Cyclosporin inhibits the MPT by binding to cyclophilin D (CypD). We hypothesize that MPT formation occurs when its proteins are in a specific conformation maintained by CypD, and MPT inhibition due to CypD deletion would hinder Ca²⁺-release and elevate matrix Ca²⁺ (Ca²⁺_m). Consistent with increased Ca²⁺_m, we find that

the activities of Ca²⁺-activated NADH dehydrogenases and matrix NADH/NAD⁺ levels are elevated in CypD^{-/-} mice. Clues to MPT identity and its physiological role were investigated by examining changes occurring with CypD deletion. We utilized proteomics to identify differences between WT and CypD^{-/-} mice. CypD^{-/-} mitochondria showed a ~40% increase in a 6.8 kDa mitochondrial proteolipid, which associates with F₁F₀-ATP synthase. ATP synthase activity is modulated by CypD and is a potential MPT candidate. CypD^{-/-} mitochondria also had a ~15% decrease in the 2-oxoglutarate/malate carrier, which can have pore-like qualities. Of particular interest was a decrease in the phosphate carrier (PiC), which binds to CypD and is suggested to be a critical MPT component. Interestingly, the PiC and ATP synthase have been shown to form a complex. Taken together, these results support the hypothesis that CypD may facilitate the formation of a complex that promotes the MPT and that the MPT can serve as a Ca²⁺-release mechanism.

A New synthetic route to CCF2/AM: An Imaging Agent for the Detection of Single Cell Protease Activity *in situ* and Pharmacological Inhibition Studies. H. Wu, Z-D. Shi, C. Li, Y. Hu, G. L. Griffiths, B. M. Connolly and T. H. Bugge. The Imaging Probe Development Center at NHLBI in collaboration with NIDCR

Dysregulated extracellular proteolysis is critical for the genesis or progression of human diseases such as myocardial infarction, stroke, rheumatoid and osteoarthritis, periodontal disease, bacterial infection, and tumor progression. Therapeutic manipulation of extracellular proteolysis for treatment of complex diseases has not been always successful and there is a paucity of agents for visualization studies of individual protease activity. Recently a membrane-permeable, fluorogenic imaging agent comprising the β -lactamase substrate: coumarin cephalosporin fluorescein acetoxymethyl ester (CCF2/AM) has been developed. However its practical applications in fluorescence microscopy, flow cytometry and fluorescent plate reader technology, and especially in automation and high-throughput screening analysis have been impeded by its unavailability. This in turn is because the synthesis of the imageable substrate has proven to be too challenging in part due to its sensitivity toward bases, acids and light during the synthesis. Here we report an alternative route to a previously patented but poorly described method for the total synthesis of CCF2/AM. This alternative synthetic route afforded satisfactory yields for most steps. The target compound was characterized by NMR and mass spectroscopy, as well as by an *in vitro* imaging assay with comparison to the authentic compound. This new route is a convenient method for the synthesis of CCF2/AM. Final yield optimizations for a couple of the synthetic steps are ongoing at IPDC.

An Apolipoprotein E Mimetic Peptide Inhibits Airway Hyper-reactivity in a House Dust Mite Model of Allergic Asthma. X. L. Yao, K. Fredriksson, Z. X. Yu, X. L. Xu, N. Raghavachari, K. J. Keeran, G. J. Zwicke, M. J.A. Amar, A. T. Remaley, and S. J. Levine, Pulmonary and Vascular Medicine Branch, Pathology Core Facility, Gene Expression Core Facility, and Laboratory of Animal Medicine and Surgery

Severe asthma is associated with the persistent expression of key disease severity genes despite treatment with corticosteroids. We used genome-wide expression profiling of the lung transcriptome to identify the up-regulated expression of apolipoprotein E (apoE) in a house dust mite (HDM) model of murine asthma following corticosteroid treatment. We also found that HDM-

challenged apoE^{-/-} mice have increased airway hyperreactivity (AHR) and mucin gene expression as compared to wild-type mice, whereas indices of airway inflammation were not affected. Here, we assessed whether reconstitution of the apoE deficiency in HDM-challenged apoE^{-/-} mice with an apoE mimetic peptide can rescue the phenotype of enhanced AHR and goblet cell hyperplasia. An apoE(130 – 149) mimetic peptide, corresponding to the low density lipoprotein (LDL) receptor-binding domain, or a control scrambled peptide, were administered to apoE^{-/-} mice via an osmotic mini-pump prior to the induction of allergic asthma by daily nasal HDM challenge for 5 days per week for 5 weeks. The apoE(130-149) mimetic peptide completely inhibited the induction of AHR and goblet cell hyperplasia, while mRNAs encoding MUC5AC and CLCA3 were significantly reduced. Administration of the apoE(130-149) mimetic peptide also significantly inhibited the induction of allergen-mediated airway inflammation and IgE production. We next assessed whether the apoE(130-149) mimetic peptide could be effective for the treatment of established asthma. Wild-type A/J mice that received nasal HDM for 5 days/week for 6 weeks were treated with the apoE(130-149) mimetic peptide during weeks 4 to 6. Treatment with the apoE(130-149) mimetic peptide reduced AHR to levels similar to that of control mice. Similarly, administration of the apoE(130-149) mimetic peptide significantly reduced mRNA levels of MUC5AC, CLCA3, IL-13 and IL-17A, but did not inhibit airway inflammation. These results demonstrate that administration of an apoE mimetic peptide rescues the phenotype of asthmatic apoE^{-/-} mice and inhibits the induction of asthma. Furthermore, treatment with the apoE(130-149) mimetic peptide completely reversed AHR and attenuated mucin gene expression in established asthma. Taken together, these findings support the concept of developing apoE-based strategies as a new approach for the treatment of asthma.

Site Specific Fluorescent Probes of α -Synuclein Fibril Assembly. T. L. Yap, C. M. Pfefferkorn, and J. C. Lee, Laboratory of Molecular Biophysics

α -Synuclein (α -syn) is a 140-residue, cytoplasmic and membrane-associated protein localized in the presynaptic terminals of neuronal cells. Upon aggregation, α -syn undergoes large conformational change from a disordered monomer to form highly ordered, β -sheet containing fibrillar aggregates (amyloid). Importantly, amyloids have been shown as the major constituent in Lewy bodies, pathological hallmarks of Parkinson's disease. Generally, it is thought that the α -syn fibril core is composed of residues 30–100; however, the molecular details of fibrillar structure remains elusive especially pertaining to the involvement of the N- and C-terminal regions. We have prepared single-Cys mutants derivatized with an environment sensitive dansyl fluorophore at specific residues spanning across the α -syn sequence (G7C, V26C, V51C, V77C, L100C, and Y136C) and characterized both soluble and aggregated forms by measurements of steady-state and time-resolved fluorescence. In order to unravel the role of these individual residues, we also utilized these fluorescent variants to monitor the fibril assembly kinetics of the wild-type protein (1.5 Dns-protein: 100 wild-type). Specifically, we find that the N- and C-terminally labeled sites are the most sensitive to fibril formation (Dns7 > Dns136 > Dns51 ~ Dns100 > Dns26 >> Dns77). Furthermore, our data suggest that residue 77 may be critical for fibril elongation.

Actomyosin Contraction Drives TCR Microcluster Movement at the pSMAC and Formation of cSMAC at the IS in T cells. C. Yi, X. Wu, J. Hammer, Laboratory of Cell Biology

The contact area between T cells and antigen presenting cells (APCs) is organized into a bull's eye arrangement of segregated concentric regions, termed the immunological synapse (IS). The center area of the IS, known as the central supramolecular activation cluster (cSMAC), is marked by accumulation of T cell receptor microclusters, while the surrounding regions, known as the peripheral SMAC (pSMAC) and distal SMAC (dSMAC), are marked by accumulations of the $\alpha 2$ integrin, LFA-1 and CD45, respectively. Although it is known that IS formation is actin-dependent, the underlying mechanism behind microcluster movement and SMAC formation is poorly understood. Here we show that a lamellapodial network of actin characterized by rapid actin retrograde flow exists at the dSMAC region, and that a lamellar network of slower actomyosin arc contraction exists at the pSMAC region of the IS. The rate of TCR microcluster movement at the dSMAC and pSMAC corresponded to the rate of actin retrograde flow and actomyosin arc contraction, respectively. Furthermore we observed continuous contraction of actomyosin arcs at the pSMAC which "swept" TCR microclusters across the region and accumulated them into an organized cSMAC at the IS center. Inhibition of myosin IIA by blebbistatin treatment blocked contractile actomyosin arc formation, reduced TCR microcluster movement at the pSMAC, and resulted in the lack of an organized cSMAC at the IS. In contrast to TCR microclusters, however, we found that the movement of ICAM-1, ligand for LFA-1, was not driven by actomyosin contraction and that a concentric pSMAC region formed even after treatment with blebbistatin. These results indicate that actomyosin contraction is a major force behind IS formation, responsible for TCR microcluster movement at the pSMAC and accumulation of TCR microclusters into an organized cSMAC at the IS.

Increased Sulfation In Chondroitin Sulfate Proteoglycans With Potential Functional Significance Following Controlled Cortical Injury In Mice. J. Yi¹, Y. Katagiri¹, D. Figge¹, P. Yu¹, A. Symes², and H. Geller¹,¹Developmental Neurobiology Section, ²Department of Pharmacology, Uniformed Services University of the Health Sciences, Bethesda, Maryland

Chondroitin sulfate proteoglycans (CSPGs) have shown to play a pivotal role in many neuronal growth mechanisms including axon guidance and possible modulation of repair processes following injury to the spine or head. Further, it has been shown that a specific sulfation pattern on the glycosaminoglycan chains may govern their inhibitory action on axonal regeneration. To date, the role of CSPGs and their sulfation pattern has not been characterized in traumatic brain injury (TBI). Controlled cortical impact injury of mild to moderate severity was performed over the left sensory motor cortex in mice. Brains were transcardially perfused and serially sectioned for immunohistochemistry at varying times post injury. Mild to moderate trauma resulted in temporally and spatially selective increases in chondroitin sulfate immunolabeling using an anti-chondroitin sulfate antibody, CS-56. These alterations in staining were observed near the contusion 'border' as early as 5 days, and persisted through 7 days post injury. The increased immunoreactivity was abrogated when sections were pre-incubated with Chondroitinase ABC. Further examination using antibodies specific for different sulfation patterns showed disparate changes following TBI. Immunofluorescence double labeling with GFAP displayed temporal overlap with the increase in chondroitin

sulfate staining. In summary, dynamic changes in sulfation pattern occur as result of brain injury. Further investigative effort is warranted to understand their significance in functional recovery after TBI.

Apolipoproteins, Serum Amyloid A, and Plasminogen: Proteomically Identified Potential Contributors to Pathogenesis of Sickle Cell Disease-Associated Pulmonary Hypertension. S. Yuditskaya, G. J. Kato, Pulmonary Vascular Medicine Branch

Pulmonary hypertension (PH) is a major complication and independent risk factor for death among adults with sickle cell disease (SCD). Using surface-enhanced laser desorption/ionization time of flight mass spectrometry (SELDI-TOF MS), we searched for biomarkers of PH in plasma specimens from 27 homozygous sickle cell anemia patients with PH and 28 without PH. In PH patients, analysis consistently showed lower abundance of a 28.1-kDa peak ($P < .001$), identified by high-resolution mass spectrometry as the oxidant-scavenging protein apolipoprotein A-I (apoA-I), which correlated with clinical assays of apoA-I ($r = .58$, $P < .001$) and high-density lipoprotein (HDL) levels ($r = .50$, $P = .001$). The PH cohort was further characterized by high levels of apolipoproteins A-II and B. Tentative identifications suggest high levels of serum amyloid A-I, and low levels of plasminogen. Immunoassays are under way to corroborate these latter two assignments. Increased PAI-1 activity promotes pulmonary vascular fibrin deposition (Pinsky et al. JCI 1998) and may be related to pathogenesis of PH in SCD. Our finding of low plasminogen in SCD-associated PH suggests a similar effect by which lower plasmin levels and thus decreased fibrinolysis results in increased fibrin deposition. Validation of proteomically identified low plasminogen levels in our PH cohort is planned with immunoassay.

Mouse Models of Human MYH9-related Diseases. Y. Zhang¹, M. A. Conti¹, P. Zervas², S. Kawamoto¹, C. Liu³, J. Kopp⁴, R. S. Adelstein¹, ¹Laboratory of Molecular Cardiology, ²Division of Veterinary Resources, ³Transgenic Core facility, ⁴Kidney Disease Section

Humans with point mutations in *MYH9*, the gene encoding nonmuscle myosin heavy chain IIA (NMHC IIA), can manifest macrothrombocytopenia, granulocyte inclusions, kidney disease [focal segmental glomerulosclerosis (FSGS), glomerular basement membrane abnormalities], cataracts, and sensorineural deafness. To gain insight into the pathological mechanism of MYH9-associated diseases, we generated mouse models of two disease-associated mutations, Arg702Cys and Asp1424Gln, using homologous recombination to replace wild type NMHC II-A with the mutant isoforms. We chose these two mutations because the Arg702Cys mutation is present in the amino-terminal motor domain of myosin IIA, while the Asp1424Gln mutation is located in the carboxyl-terminal rod domain, which regulates myosin filament formation. Heterozygous Asp1424Gln mutant mice produced homozygous mutant offspring at close to normal ratios. By contrast, homozygous Arg702Cys mice die between embryonic day (E)8.5 and 10.5, which is considerably later in development than knockout myosin II-A mice (E6.5). These results indicate that the motor domain function of NMHC IIA is critically important during mouse embryonic development. Interestingly, giant platelets were found in the blood smears from both Arg702Cys and Asp1424Gln adult heterozygous mice. The homozygous Asp1424Gln adult mice have even larger platelets and an abnormally low platelet count. Some but not all adult heterozygotes of both mutant lines have higher urine albumin/creatinine ratios at 8-9 weeks, suggest-

ing that glomerular disease is developing in these mice. Light and transmission electron microscopy studies show FSGS in the heterozygous Arg702Cys and both the heterozygous and homozygous Asp1424Gln mice, while the basement membranes appear normal. Our preliminary results suggest that these mouse models should be useful in understanding the pathophysiology of human *MYH9*-related diseases.

Methionine Sulfoxide Reductase A Overexpression in Murine Embryonic Fibroblast and Hepatocyte does not Improve Cellular Resistance Against Oxidative Stress. H. Zhao, G. Kim, C. Liu, R.L. Levine; Lab of Biochemistry

Methionine residue in proteins is highly susceptible to reactive oxygen species, forming either R or S form of methionine sulfoxide (MetO). However, methionine oxidation is reversible with enzyme methionine sulfoxide reductase A (msrA) which is specific for the reduction of S form MetO, while msrB being specific for the R form. It has been suggested that msrA functions as a cellular antioxidant by scavenging ROS through cyclic oxidation and reduction of methionine residues in proteins. Since mitochondria are considered major source of ROS and in mammalian cells msrA has been found in both cytosol and mitochondria, it is of particular interest whether msrA overexpression in mitochondria would enhance cellular defense against oxidative stress. In this study, transgenic mice with msrA overexpression in either mitochondria or cytosol were developed. Murine embryonic fibroblasts (MEF) derived from these mice showed good correlation between msrA protein level and msrA enzymatic activity. In contrast, non-transgenic MEF had no detectable endogenous msrA and served as a control. However, cell viability assay revealed that transgenic MEFs were no more resistant to various oxidizing agents than the non-transgenic MEF was. Further investigation showed that msrA overexpression MEF had very low or undetectable levels of thioredoxin (Trx) and thioredoxin reductase (TrxR), which was in sharp contrast to those from hepatocytes. Cell viability after hydrogen peroxide treatment in hepatocytes derived from transgenic mice didn't differ from those isolated from msrA knockout mice, but significantly higher in those from wild type mice. The results suggest: 1) msrA may not be physiologically indispensable to MEF, since its endogenous level is not detectable; 2) The antioxidant role of msrA is dependent on the integrity of complete msrA-Trx-TrxR system. Deficiency in Trx and TrxR in transgenic MEF probably abrogates the defense msrA might offer against oxidative stress. 3) While knocking out msrA in hepatocytes decreases cellular defense against oxidative stress, msrA overexpression does not necessarily improve stress resistance.

Forkhead-dependent Mesodermal Gene Regulation in *Drosophila*. X. Zhu¹, B. W. Busser¹, S. M. Ahmad¹, L. Shokri², S. S. Gisselbrecht², A. Aboukhalil², A. Haimovich¹, M. L. Bulyk², and A. M. Michelson¹, ¹Genetics and Developmental Biology Center, ²Division of Genetics, Department of Medicine, Brigham and Women's Hospital, Boston, MA

Forkhead (Fkh) proteins comprise a large family of conserved transcription factors (TFs) with diverse developmental functions. The regulatory roles of this family in *Drosophila* have been largely unexplored, although the expression of Fkh TFs in various subsets of embryonic mesodermal cells suggests that they may control cell fate specification or differentiation. To investigate the potential involvement of these TFs in the regulation of cardiac gene expression, we took an integrated approach combining computational methods with cis and trans tests of gene regula-

tion. We first performed genome-wide computational scans which revealed a statistical enrichment of Fkh binding sites clustered together with motifs for a core set of known and candidate heart TFs within the non-coding sequences of a group of genes expressed in the heart. We next mutated the evolutionarily conserved canonical Fkh binding sites in an enhancer from the *nidogen* (*ndg*) gene that we previously had demonstrated is active in the heart, somatic muscle founder cells (FCs) and gut musculature. These mutations resulted in de-repression of the reporter in cardiac and pericardial cells of the heart, as well as in fusion competent somatic myoblasts that do not normally express *ndg*. In contrast, elimination of Fkh binding sites caused a loss of *ndg* enhancer activity in the visceral mesoderm (VM). To determine the specific Fkh TFs responsible for these effects, we next determined the mesodermal expression of every *Drosophila* Fkh domain-encoding gene in relation to *ndg*. These results led us to a set of candidate regulatory TFs that could account for the differential effects seen in each mesodermal cell type. We are currently investigating the role of each of these TFs in regulating *ndg* gene expression using a combination of genetic and RNAi-based strategies. In initial experiments, loss-of-function mutations in *jumeau* (*jumu*), which encodes a Fkh TF expressed in the heart and a subset of FCs, caused a similar de-repression of *ndg* in the heart as observed with the Fkh mutation in the *ndg* enhancer. In addition, loss-of-function of *biniou* (*bin*), which encodes a Fkh TF having VM expression, was associated with reduced *ndg* expression in the VM, similar to the effect on VM activity of the Fkh site mutation in the *ndg* enhancer. These results suggest that different tissue-specific Fkh TFs mediate distinct gene expression responses through the same binding sites in a single enhancer, and support a role for these factors in determining the unique genetic programs that characterize different subtypes of mesodermal cells.

Characterization of the Interaction between Zona Occludens-2 and Sorting Nexin 27 in Kidney Epithelial Cells. S. Zimmerman, A. Udofa, M. Playford, S. Milgram Laboratory of Kidney and Electrolyte Metabolism

The Sorting Nexin (SNX) superfamily of proteins are characterized by the presence of a phox-homology (PX) domain. SNX proteins and associate with thebind phosphatidylinositol-3-monophosphate (PtdIns3P) rich regions of the early endosome. Our lab previously showed that SNX27 interacts with the COOH-terminus of the cystic fibrosis transmembrane conductance regulator (CFTR) through the SNX27 PDZ domain. To further identify SNX27-interacting proteins, we used a GST-SNX27 PDZ domain fusion protein as the "bait" in a mouse kidney epithelial cell lysate and identified interacting proteins using mass spectrometry. Here we show a novel interaction between SNX27 and zona occludens protein 2 (ZO2, TJP2). ZO2 is known as a tight junction protein but has been shown to have a second localization and function in the nucleus. How ZO2 shuttles between these two subcellular compartments is unknown. We used GST pulldowns and immunoprecipitation assays to confirm the interaction between the PDZ domain of SNX27 and the PDZ binding motif of ZO2. We also performed preliminary confocal microscopy experiments to examine the distributions of ZO2 and SNX27. We find that SNX27 and ZO2 colocalized on endosomal vesicles but not at the tight junction. These results suggest that SNX27 may be involved in trafficking of ZO2 to or from one of its functional locations.

Potential Roles of PDE3B Knockout in Acquisition of Brown Fat Characteristic by White Adipose Tissue in Mice.

E. Zmuda-Trzebiatowska, V. Manganiello, Translational Medicine Branch

The cAMP-degrading enzyme, phosphodiesterase 3B (PDE3B) is an important regulator of energy metabolism. PDE3B knockout (KO) mice in SvJ129 background have less white adipose tissue (WAT) than their wild type (WT) counterparts. Furthermore, their white fat shows characteristics of brown adipose tissue (BAT), including expression of UCP1, as well as increased oxygen consumption, mitochondrial biogenesis and fatty acid oxidation. This phenotype is less pronounced in PDE3B KO mice in C57/BL6 background which is prone to obesity. In order to dissect genes involved in acquisition of brown fat characteristics by WAT, we have studied genetic and protein profiles of both PDE3B KO strains. Thus, expression of several genes related to BAT, e.g. PRDM16, PPAR α and glycerokinase, was elevated in PDE3B KO as compared to WT in both strains. However, several changes noted in SvJ129 PDE3B KO, including increased expression of BAT-genes, e.g. PGC1 α (PPAR γ coactivator 1 α) and Elovl3 (elongation of very long chain fatty acids), as well as decreased expression of WAT-genes, e.g. CtBP (C-terminal Binding Protein), RIP140 and p107, were not seen in C57/BL6 PDE3B KO. C57/BL6 mice were treated with the β 3-adrenergic agonist, CL316243, to find out whether additional increase in cAMP levels can enhance brown fat phenotype in their WAT. Our results suggest that, in C57/BL6 mice, CL316243 treatment leads to reduced expression of genes related to both WAT and BAT differentiation program (PRDM16, CtBP, RIP140, p107), while increasing expression of genes and proteins related to fatty acid oxidation, mitochondrial biogenesis and thermogenesis (PGC1 α , PPAR δ , UCP1, Elovl3).

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